JOURNAL OF AGRICULTURAL RESEARCH

VOL. XXVIII WASHINGTON, D. C. MAY 10, 1924 No. 6

FACTORS INFLUENCING THE BINDING POWER OF SOIL COLLOIDS[1]

By HOWARD E. MIDDLETON

Scientist in Soil Physical Investigations, Bureau of Soils, United States Department of Agriculture

INTRODUCTION

In a previous publication by Moore et al. (*7*),[2] of this Bureau, the point was brought out that the colloidal material is evidently the principal binding material of the soil. Further study has not only confirmed this point but, as shown by Anderson et al. (*1*), approximately 95 per cent of the total absorption of the soil is due to the soil colloids. The influence of the soil colloids on the physical and chemical properties of soil is no doubt coming to be one of the most important studies in soil science. The binding power of the soil colloids is of particular importance as regards tillage operations, foundations, and the construction of dams and roads. With these points in mind this investigation was undertaken in order to determine if possible the factors influencing the binding power of the soil colloids and the relation of the compressive strength of the soil to the amount of colloidal material present in the soil.

PREVIOUS WORK

Various methods for the determination of the binding power of soils have been described. The methods of Atterberg (*3*) and Puchner (*8*) are probably the more important. These methods are based primarily on the breaking strength of briquettes. Marquis (*6*) describes various methods for determining the binding power of soils, particularly those of Atterberg and Puchner. He found very wide discrepancies in the results by each of these methods. More recently Arnd (*2*) has described a method by which he determines the binding power by friction, rotating a cylinder of soil against a stationary prism of soil under a definite pressure. None of these methods, however, takes into account the colloidal content of the soil.

Jackson (*5*) has described a method for determining the binding power of rock powder, which was modified somewhat and used in this investigation.

METHOD OF INVESTIGATION AND APPARATUS

For the purposes of this investigation the material[3] under consideration was made up into briquettes 25 mm. in diameter and 25 mm. high under a pressure of 2,000 pounds per square inch, with a certain predetermined amount of water, carefully dried, heated 18 hours at 110° C., cooled in a desiccator and subjected to the compression test in an Olsen universal testing machine.[4] The load

[1] Received for publication March 24, 1924.

[2] Reference is made by number (*italic*) to "Literature cited," p. 513.

[3] "Material" as used in this paper refers to soil or to mixtures of soil colloids and sand.

[4] The use of this machine was granted for this purpose by the Division of Tests, Bureau of Public Roads, U. S. Dept. of Agriculture.

indicated by the machine at the point of failure of the briquette was taken as its breaking strength. The average of three or four such determinations was taken in each instance. The dry weights of the briquettes varied considerably with different materials; so for comparative purposes, the average load was divided by the weight of the briquette, giving the load per gram of material. By dividing the load per gram of material by the amount of colloid in one gram of the material, the load per gram of colloid was determined.

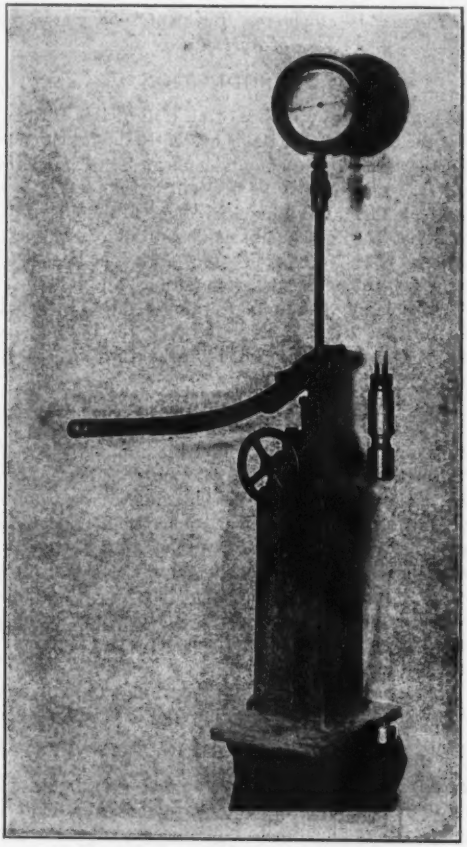


FIG. 1.—Hydraulic pump used in molding briquettes

For the purpose of forming the briquettes a hydraulic pump (fig. 1) was used. A brass cylinder (fig. 2) was drilled with a 31/32-inch drill, bored to 63/64 inch and lapped out with a lead lap. This made the diameter of the opening 25 mm. A steel piston was turned out so that it slipped easily into this opening and was made water tight at its lower end with a leather washer. A small auxiliary cap was made to fit the opening at the top of the cylinder and a larger cap made to screw on over this. The purpose of the small cap was to protect the surface of the briquette when the outer cap was removed. The cylinder was attached to the pump, as shown in figure 1.

In making a briquette the piston was pushed to the bottom of the cylinder with a wooden stick, the material was introduced into the cylinder, and the caps put in place. The valve on the pump was closed and the pressure gradually raised to 2,000 pounds, as indicated by the gauge. After the pressure had been applied a sufficient length of time, it was released by opening the valve and the caps were removed. Then the valve was closed and the briquette pushed out of the cylinder (as shown in fig. 2) by operating the hydraulic pump, and removed.

All briquettes which were used for compression tests were made 25 mm. high. In order to determine the amount of material necessary to make a briquette 25 mm. high, a preliminary briquette containing 20 gm. of the material was made up. The height of this briquette was measured with a micrometer and the amount of material necessary to make a briquette 25 mm. high calculated by simple proportion. This preliminary briquette was then used to determine the amount of moisture in the material at the time the briquettes were made.

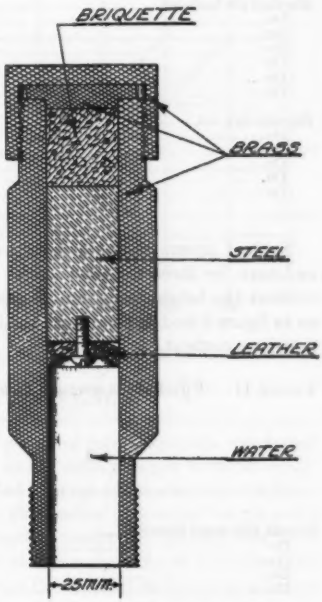


FIG. 2.—Cylinder for molding briquettes

GENERAL CONSIDERATIONS

At the beginning of this work, it was found very difficult to get determinations of the breaking strength of any one given material which could be duplicated at two different times. The briquettes in a given series made up at one time would give very different results from the same series made up at another time. In tracing out the causes of the discrepancies there were found to be four main factors governing the compressive strength of the briquettes, as follows:

(*a*) The amount of moisture present in the material when the briquettes were made.

(*b*) The treatment of the material before the briquettes were made.

(*c*) The pressure used in making up the briquettes.

(*d*) The method of drying the briquettes.

When these factors were taken into consideration the results could be duplicated within 5 per cent of the average load.

MOISTURE CONTENT

Dry briquettes of a given material varied in breaking strength depending on the moisture content of the material. By varying the moisture content and keeping the dry weight of the material constant a point was found where the briquette reached a minimum size. This indicated that the density of the material was greatest at this point. For the purpose of this investigation, the moisture content at this point was called the critical moisture content. Since the diameters of the briquettes were held constant by the size of the opening in the cylinder, the differences in the size of the briquettes were indicated by their heights.

TABLE I.—*Effect of the amount of moisture on the height of the briquette*

Soil	Weight of wet briquette	Weight of dry briquette	Per cent moisture	Height of briquette	Height of briquette per gram of dry soil
	Grams	*Grams*		*Millimeters*	*Millimeters*
Norfolk fine sandy loam soil	20.95	20.00	4.8	23.27	1.163
Do	21.48	19.99	7.5	22.75	1.138
Do	21.99	20.02	9.8	22.47	1.122
Do	22.47	20.03	12.2	22.50	1.123
Do	22.75	20.11	13.1	22.85	1.136
Marshall silt loam soil	20.97	19.90	5.4	26.60	1.337
Do	20.96	19.45	7.8	25.03	1.287
Do	21.96	19.93	10.2	24.74	1.242
Do	22.37	19.82	12.9	23.96	1.210
Do	22.95	19.99	14.8	23.75	1.189
Do	23.45	19.92	17.7	24.15	1.212
Do	23.94	19.90	20.3	25.27	1.270
Sharkey clay soil	21.78	20.00	8.9	26.63	1.332
Do	22.53	19.93	13.0	24.71	1.240
Do	23.12	19.95	15.9	23.95	1.200
Do	23.57	19.95	18.1	23.88	1.196
Do	23.78	19.92	19.4	24.17	1.213
Do	24.34	19.94	22.1	25.17	1.262

Table I shows how the height of the briquette varies with the amount of moisture for three different soils. In order to determine the critical moisture content the height per gram of dry soil is plotted against the per cent moisture as in figure 3 and the value at the lowest point of the curve indicates the critical moisture content.

TABLE II.—*Effect of the amount of moisture on the breaking strength of the briquette*

Soil	Moisture	Weight of briquette	Average load per briquette	Load per gram of soil
	Per cent	*Grams*	*Kilograms*	*Kilograms*
Norfolk fine sandy loam soil	4.8	21.6	88	4.1
Do	7.5	22.0	143	6.5
Do	9.8	22.3	175	7.8
Do	12.2	22.3	161	7.2
Do	13.1	22.1	141	6.4
Marshall silt loam soil	5.4	18.7	102	5.5
Do	7.8	19.5	213	10.9
Do	10.2	20.2	351	17.4
Do	12.9	20.7	528	25.5
Do	14.8	21.1	671	31.8
Do	17.7	20.7	673	32.5
Do	20.3	19.8	471	23.8

The curves indicate a critical moisture content for the Sharkey clay soil of 17½ per cent, for the Marshall silt loam soil of 15½ per cent, and for the Norfolk fine sandy loam of 11 per cent. If greater accuracy than one-half of 1 per cent was desired, the briquettes were made up near the critical moisture content with increments of one-half of 1 per cent moisture and a new graph plotted. This method showed the critical moisture content for the Norfolk fine sandy loam soil, for example, to be at 11.3 per cent.

Briquettes of the Norfolk and Marshall soils were made up of the same samples of material used in the determination indicated in Table I to determine the effect of the amount of moisture on the breaking strength. These results are shown in Table II and graphically in figure 4. In this case, since the briquettes

were all of the same size, the weight of the briquette is taken as a measure of the density. It may readily be seen from figure 4 that the maximum load corresponds very closely to the maximum density of the briquette at the critical moisture content. Since the maximum load occurs at the critical moisture content, in comparing the breaking strength of two or more materials, each is made up at its critical moisture content.

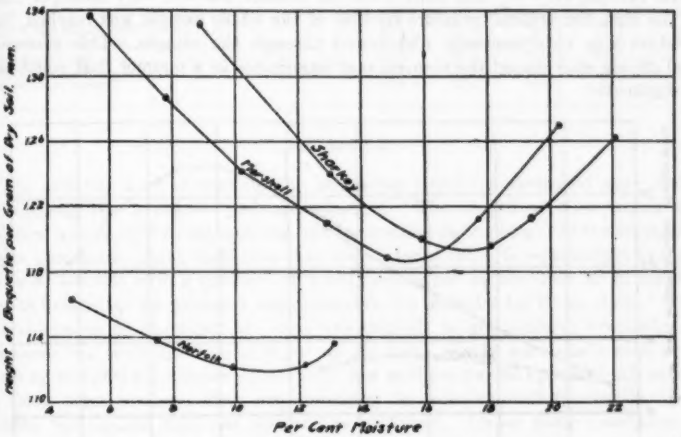


FIG. 3.—Showing the effect of the amount of moisture on the height of the briquette

TREATMENT OF THE MATERIAL

The treatment of the material before being made up into briquettes was found to be one of the most important factors as well as the most difficult to standardize. In order to get the water thoroughly distributed through the material a considerable amount of mixing was necessary, and in the matter of mixing the personal equation entered to a large extent. A mechanical method of mixing was tried out with but little success due to the fact that it was very difficult to control the amount of moisture in the mixture accurately, and the method could not be adapted to the small amounts of material available.

The method which was most easily duplicated in comparative tests of soils was to weigh out sufficient air-dry soil to make 100 gm. on the oven-dry basis. The soil was weighed out in a 250 cc. beaker and sufficient water added to bring it slightly above the critical moisture content. The material was then transferred to a glass mortar and rubbed gently with a glass pestle until the water was evenly distributed. It was then replaced in the beaker and allowed to stand in a desiccator containing distilled water for 42 hours. At the end of this time the material was weighed, the moisture content adjusted if necessary and the briquettes were made up as described above. This was designated as the first method.

The second method was the same as the first up to the point where the water was added. In this case the water was added gradually, the material being stirred with a spatula until sufficient water had been added to bring it to the consistency of thick cream. The material was then allowed to dry in the air with occasional stirring until it reached the critical moisture content. It was then transferred to the glass mortar and treated in the same manner as in the

first method. This method was found to give much higher results than the first method. This was probably due to the fact that the colloidal material was more thoroughly broken up and dispersed. However, the results were less uniform and very difficult to duplicate due to the fact that the same degree of dispersion was difficult to attain in two different samples of the same material. Another difficulty was encountered in drying the material. At the surface it would become very dry and hard while the interior was still very moist, so that by the time the critical moisture content of the whole sample was reached, the moisture was very unevenly distributed through the sample. This excessive local drying also caused the formation of aggregates in a manner that could not be duplicated.

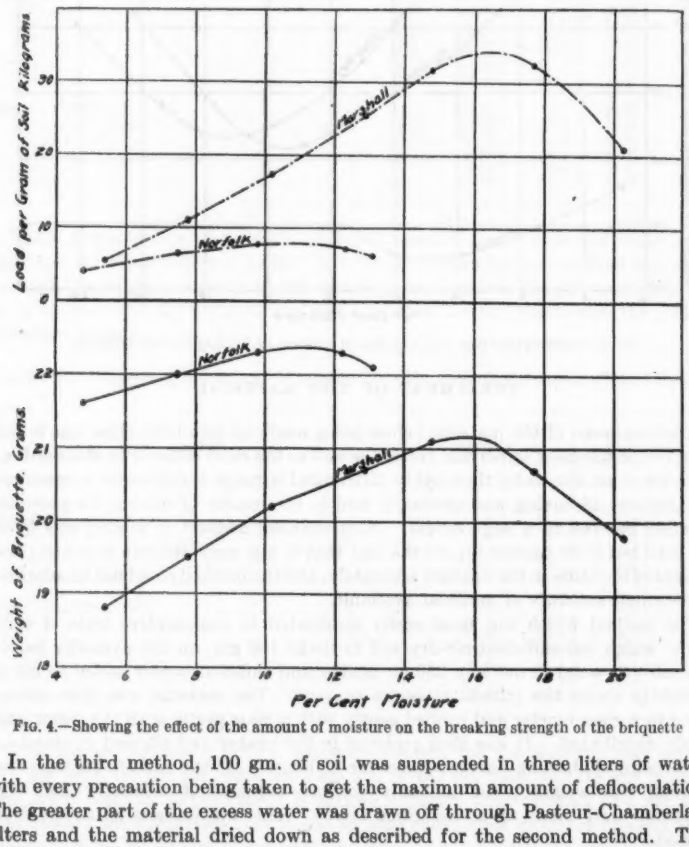


FIG. 4.—Showing the effect of the amount of moisture on the breaking strength of the briquette

In the third method, 100 gm. of soil was suspended in three liters of water, with every precaution being taken to get the maximum amount of deflocculation. The greater part of the excess water was drawn off through Pasteur-Chamberlain filters and the material dried down as described for the second method. This method consumed a large amount of time and on the two samples tried did not give results very greatly different from the second method. No attempt was made to duplicate the results by this method.

Table III shows the results obtained by the three different methods of treatment on two soils and by the first and second method on five soils. Since the first method could be duplicated with greater reliability in addition to requiring a shorter time to carry out, it was adopted as a standard.

TABLE III.—*Effect of method of treatment on the breaking strength of the briquette*

Soil	Colloid	Moisture	Load per gram of soil		
			First method	Second method	Third method
	Per cent	*Per cent*	*Kilograms*	*Kilograms*	*Kilograms*
Norfolk fine sandy loam soil	10.7	11.4	5.9	10.1	9.6
Marshall silt loam soil	27.3	16.0	30.7	37.5	39.8
Sharkey clay soil	61.2	18.0	75.2	103.8	.....
Ontario loam soil	18.5	14.0	12.8	19.6	.....
Ontario loam subsoil	11.4	12.5	16.2	31.1	.....

PRESSURE

The pressure used in making the briquettes could be controlled very easily by keeping the hydraulic pressure constant. The length of time which the pressure was applied to the material had some effect on the height of the briquette. Tests were made which indicated that the material came to equilibrium in from 30 to 60 seconds with a pressure of 2,000 pounds per square inch, so in making up the briquettes the pressure was applied to the material for 60 seconds. With the apparatus as described above it was difficult to get uniform briquettes at pressures less than 2,000 pounds per square inch. Tests were made with pressures up to 5,000 pounds per square inch, but as there was no apparent advantage in using more pressure than was necessary in securing uniform results, 2,000 pounds per square inch was adopted as standard. Under these conditions it was possible to make up four briquettes of equal quantities of a given material which would be within 0.05 mm. of the same height.

DRYING THE BRIQUETTES

Considerable difficulty was encountered in drying the briquettes so as to avoid cracking and checking. By removing the moisture very slowly and uniformly at the beginning of the drying this difficulty was largely overome. It was found that after the moisture content had been reduced approximately 5 per cent the briquettes could be dried quite rapidly without danger of cracking. The method employed was to allow the briquettes to stand in a desiccator containing distilled water for 24 hours after being made up. At the end of this time they were transferred to an electrical drying oven, which was allowed to remain at room temperature and through which a very slow current of air nearly saturated with moisture was passed for 24 hours. Then the temperature of the oven was raised to 30° C. and the process continued 24 hours longer. The air was then shut off and the temperature of the oven raised to 110° C. for 18 hours. The briquettes were then transferred to a desiccator containing sulphuric acid and allowed to cool before being tested. Another method which gave equally as good results, but which took a little more time, was to have a series of four desiccators in which the saturation of air was, respectively, 100, 75, 50, and 25 per cent. The briquettes were allowed to stand 24 hours in each desiccator and then transferred to the drying oven at 110° C. The principal consideration in any method of drying is to so regulate the system that the moisture is supplied to the surface of the material to be dried at the same rate that it is carried off by evaporation so that the surface does not become dry and allow it to shrink and crack.

THE BINDING POWER OF SOIL COLLOIDS IN THE SOIL

The binding power of the colloid in the soil was found to depend upon a number of factors. One important factor has been discussed by Gile et al. (*4*) in a previous publication, namely, the extractable and unextractable colloid. It would seem that the portion of the colloid which is the more easily dispersed would have a greater binding power than that which is more difficultly dispersed. There is no direct evidence that this is true, but it has been noted in the case of moist and air-dry colloids, which is more or less analogous. It has been noted in this laboratory that when a colloid in the moist condition is allowed to become air dry or oven dry, it is very difficult to redisperse. This is no doubt due to the formation of aggregates in the process of drying similar to the colloidal aggregates found in the soil.

Another factor of some importance is the difficulty of determining exactly the amount of colloid in the soil. The methods of determining the amount of colloid show considerable variation in some soils, but these variations are usually of small consequence in themselves. However, if the amount of colloid assumed to be in the soil is in error by a small per cent, when this is translated into the load per gram of colloid, the error will be magnified several times.

In determining the binding power of the soil colloids in the soil 17 soils in addition to the five listed in Table III were made up into briquettes by the first method, as described on page 500 and tested in the same way. These samples were subsamples of the same material used by Gile et al. (*4*) in determining the amount of colloid, and for this purpose the amount of colloid was assumed to be that shown by the water vapor adsorption. The results of these tests are shown in Table IV. If we assume that L, the load per gram of soil in kilograms, varies as C, the amount of colloid present, then

$$L=kC^n$$

TABLE IV.—*The binding power of soil colloids in the soil*

Soil	Colloid by water vapor adsorption	Weight of dry briquette	Average load per briquette	Load per gram of soil	Load per gram of colloid
	Per cent	*Grams*	*Kilograms*	*Kilograms*	*Kilograms*
Carrington loam soil	32.4	22.1	476	21.5	66.5
Carrington loam subsoil	29.4	25.2	497	19.7	67.1
Cecil clay loam soil	10.2	24.2	179	7.4	72.5
Cecil clay loam subsoil	31.5	23.8	410	17.2	54.7
Chester loam soil	8.5	22.7	231	10.2	119.6
Chester loam subsoil	24.8	23.5	485	20.6	83.3
Manor loam soil	18.4	23.2	404	17.4	94.6
Manor loam subsoil	18.6	23.3	399	17.1	92.2
Marshall silt loam soil	27.3	21.0	644	30.7	112.5
Marshall silt loam subsoil	34.3	22.9	1,188	51.9	151.4
Norfolk fine sandy loam soil	10.7	21.9	129	5.9	55.0
Norfolk fine sandy loam subsoil	20.5	24.4	431	17.7	86.1
Ontario loam soil	18.5	21.2	272	12.8	69.4
Ontario loam subsoil	11.4	23.3	378	16.2	142.2
Orangeburg fine sandy loam soil	6.2	22.0	45	2.0	33.0
Orangeburg fine sandy loam subsoil	21.5	25.1	361	14.4	66.9
Sassafras silt loam soil	8.0	22.0	127	5.8	72.2
Sassafras silt loam subsoil	18.9	23.0	447	19.4	102.8
Sharkey clay soil	61.2	21.1	1,585	75.2	122.8
Susquehanna clay subsoil	30.4	23.8	481	20.2	66.5
Wabash silt loam soil	29.8	21.9	1,006	46.0	154.2
Wabash silt loam subsoil	32.4	22.2	1,025	46.2	142.5

Substituting the values of L and C from Table IV in this equation and solving for the values of k and n which most nearly satisfy all of the equations

$$L=0.42\ C^{1.24}$$

This equation is shown graphically in figure 5 and also the observed values from Table IV.

If we let L_c represent the load per gram of colloid in kilograms, then by definition

$$L_c=\frac{L}{C}\times 100$$

Substituting in the above equation

$$L_c=\frac{0.42\ C^{1.24}}{C}\times 100=42\ C^{0.24}$$

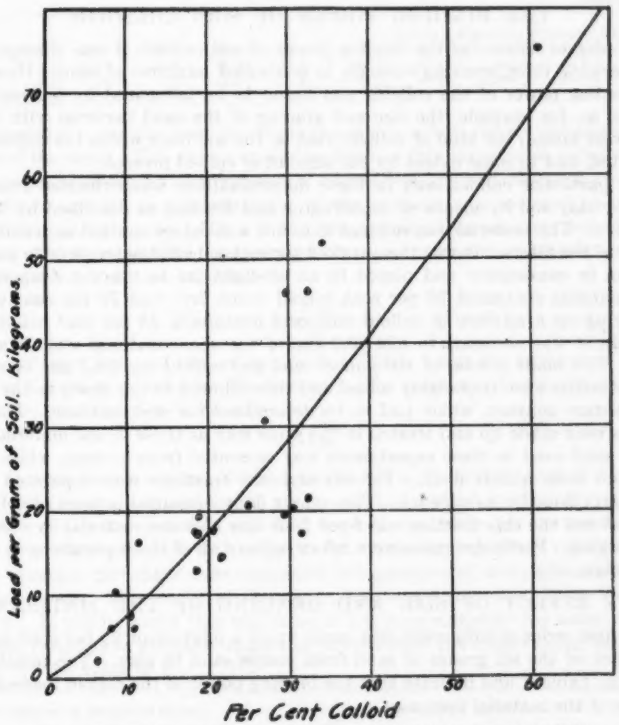


FIG. 5.—The relation between the load per gram of soil and the amount of colloid.

This indicates that under these conditions the load per gram of colloid depends upon the amount of colloid in the soil, thus

$$C=\ 1,\ L_c=\ 42$$
$$C=20,\ L_c=\ 86$$
$$C=50,\ L_c=107$$

Table IV shows this to be true in a general way, the soils with a small amount of colloid having a low load per gram of colloid and vice versa. However, the variations from the curve of the load per gram of soil as indicated in figure 4, indicate a much larger variation in the load per gram of colloid. Considering the

wide variation in the types and classes of soil, and the numerous factors affecting the binding power of the colloid, this relation seems to be very satisfactory.

It is evident from the relation between the load and the amount of colloid that the amount of colloid in the soil could be calculated provided the load were known. This is somewhat analogous to the water adsorption factor as developed by Robinson (*9*). While this method is not adaptable to as great refinement and accuracy as the adsorption methods, it would seem to indicate the colloidal properties of soils for comparative purposes, particularly from the physical standpoint, more readily than the mere knowledge of the amount of colloid in each of the different soils under comparison. By combining the two methods, as was done in working out the above relation, one may be used as a control on the other. However, for the purpose of this discussion, it was assumed that the amount of colloid in the soil was that shown by the water vapor adsorption.

THE BINDING POWER OF SOIL COLLOIDS

In order to determine the binding power of soil colloids it was thought best to determine their breaking strength in controlled mixtures of sand. However, the binding power of the colloids was found to be influenced by a number of factors, as, for example, the size and grading of the sand particles with which they were mixed, the kind of colloid, that is, the soil from which the colloid was extracted, and to some extent by the amount of colloid present.

The particular colloid used in these determinations was extracted from the Sharkey clay soil by means of centrifuging and filtering as described by Moore et al. (*7*). The material was reduced to as low a moisture content as possible by means of the filters. It was then worked through a 1-millimeter sieve to make it uniform in consistency and placed in an air-tight jar to prevent evaporation. This material contained 30 per cent colloid (oven dry) and 70 per cent water. In mixing up a mixture of colloid and sand containing 25 per cent colloid, for example, it was necessary to mix 83.3 gm. of the above material with 75 gm. of sand. This made a total of 100 gm. of sand and colloid and 58.3 gm. of water. The materials were thoroughly mixed and then allowed to dry down to the critical moisture content, which had to be determined for each mixture. The briquettes were made up and treated in the same way as those of soil material.

The sand used in these experiments was separated from a clean, white river sand and from quartz flour. The silt and clay fractions were separated from the quartz flour by subsidence. The quartz flour contained a trace of colloidal material and the clay fraction was freed from this colloidal material by repeated centrifuging. Particular pains were taken to have all of the separations as exact as possible.

THE EFFECT OF SIZE AND GRADING OF THE MATERIAL

The first series of briquettes was made up of a mixture of 25 per cent colloid with each of the six grades of sand from coarse sand to clay. The results are shown in Table V and indicate that the binding power of the colloid increases as the size of the material decreases.

TABLE V.—*Effect of the size of the material on the breaking strength of the briquettes, 25 per cent Sharkey colloid*

Material	Size of particles	Weight of dry briquette	Average load per briquette	Load per gram of briquette	Load per gram of colloid
	Millimeter	*Grams*	*Kilograms*	*Kilograms*	*Kilograms*
Coarse sand	1.0 –0.5	21.7	82	3.8	15.1
Medium sand	.5 – .25	21.4	91	4.3	17.0
Fine sand	.25 – .1	21.5	134	6.2	24.9
Very fine sand	.1 – .05	21.6	211	9.8	39.1
Silt	.05 – .005	21.4	526	24.6	98.4
Clay	.005– .001	21.5	1,325	61.6	246.6

The next series of briquettes was made up of 25 per cent colloid with three different mixtures of sand of various grades. The results are shown in Table VI and indicate that the binding power of the colloid depends upon the grading as well as the size of the material. Mixtures *A* and *B* were prepared by mixing the indicated amounts of the relative sand sizes; mixture *C* was a quartz flour, the composition of which was determined by mechanical analysis.

TABLE VI.—*Effect of the grading of the noncolloidal material on the breaking strength of the briquettes, 25 per cent Sharkey colloid*

Name	Mixture of noncolloidal grains—mechanical analyses						Weight of dry briquette	Average load per briquette	Load per gram of briquette	Load per gram of colloid
	Coarse sand	Medium sand	Fine sand	Very fine sand	Silt	Clay				
							Grams	*Kilograms*	*Kilograms*	*Kilograms*
A	30.0	25.0	20.0	15.0	10.0	0.0	24.9	526	21.1	84.6
B	10.0	15.0	20.0	25.0	30.0	.0	25.4	1,101	43.4	173.5
C	.0	.0	.3	13.3	71.0	15.4	23.8	1,690	71.0	284.0

It is natural to assume that the binding power is influenced by the amount of surface of the sand. The surface area of material of this character is very difficult to estimate accurately, but for purposes of comparison the particles were assumed to be cubes and the average diameter of the particles was assumed to be the longest diagonal of a cube. Then if D is the average diagonal measured in centimeters, and S is the total surface area of 1 gm. of material (specific gravity 2.65) measured in square centimeters

$$S=\frac{3.92}{D}^{5}$$

The surface of 1 gm. of each of the sands in Table V and the mixtures of sands in Table VI was calculated by this formula. The relation between the load and the surface was assumed to be of the form

$$L=kS^n$$

where L is the load per gram of briquette, and S is the surface area of 1 gm. of sand. For the sands separately the values of k and n which most nearly satisfied all of the equations were 0.42 and 0.52 respectively, while for the mixtures of sands they were 1.66 and 0.47 respectively. Substituting these values in the above equation, the loads were calculated for purposes of comparison with the observed loads. The results of these calculations are shown in Table VII and figure 6. It will be noted that the value of n is approximately the same in each case, the variation in strength under the two different conditions being indicated largely by the value of k.

[5] This equation is derived as follows:

The side of a cube in terms of the longest diagonal $=\frac{D}{\sqrt{3}}$.

The surface area of a cube in terms of the longest diagonal $=2D^2$.

The volume of a cube in terms of the longest diagonal $=\frac{D^3}{3\sqrt{3}}$.

The volume of 1 gram of sand $=\frac{1}{2.65}$.

The number of particles in 1 gram of sand $=\frac{\frac{1}{2.65}}{\frac{D^3}{3\sqrt{3}}}=\frac{3\sqrt{3}}{2.65D^3}$.

The total surface is equal to the number of particles multiplied by the surface area of 1 particle.

$$S=\frac{3\sqrt{3}}{2.65D^3}\times 2D^2=\frac{3.92}{D}.$$

TABLE VII.—*Effect of surface area on breaking strength of the briquettes*

(a) CALCULATED FROM TABLE V

Material	Surface area of 1 gram	Load per gram of briquette	
		Observed	Calculated
	Square centimeters	*Kilograms*	*Kilograms*
Coarse sand	52	3.8	3.3
Medium sand	105	4.3	4.8
Fine sand	224	6.2	7.1
Very fine sand	523	9.8	11.0
Silt	1,425	24.6	18.7
Clay	15,685	61.6	65.4

$L=0.42\ S^{0.81}$

(b) CALCULATED FROM TABLE VI

A	308	21.1	24.6
B	625	43.4	34.3
C	3,495	71.0	76.9

$L=1.66\ S^{0.47}$

The above values are all based on a colloidal content of 25 per cent. Sufficient material was not available, particularly in the silt and clay groups, to test this relation extensively except in a general way. The value of n was found

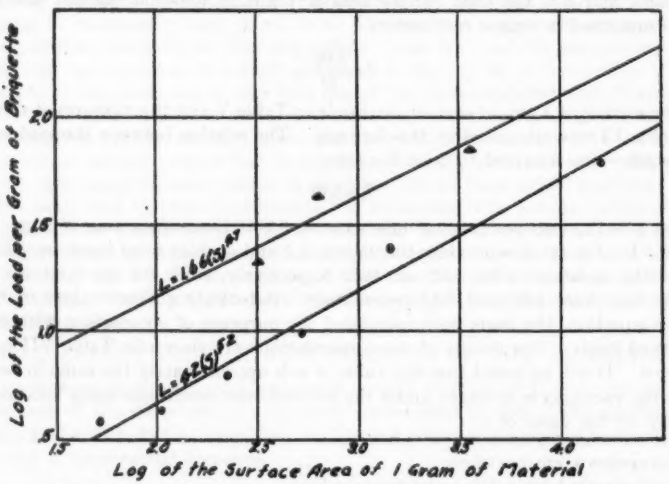


FIG. 6.—Showing the effect of the surface on the breaking strength of briquettes

to be practically constant as shown above, the variation in strength due to size and grading of the material and the amount of colloid present being indicated by variation in the value of k.

THE EFFECT OF THE AMOUNT OF COLLOID

To determine the effect of the amount of colloid present in a mixture on the binding power of the colloid, varying amounts of colloid were mixed with quartz flour and also with medium sand. The results of these tests are shown in Table VIII. It may be noted from the methods of calculating the load per gram of

colloid, that if the load per gram of briquette varied directly as the amount of colloid present, the load per gram of colloid would be constant for each material. With quartz flour this is practically the case with the exception of the first determination (50 per cent colloid). With medium sand the variation is larger. It may also be noted that the variation in the load per gram of colloid, particularly with medium sand, follows more or less closely the density of the material as indicated by the weight of the briquette.

TABLE VIII.—*Showing the effect of the amount of colloid on the breaking strength of briquettes*

Sharkey colloid and—	Per cent of colloid	Weight of briquette	Average load per briquette	Load per gram of briquette	Load per gram of colloid
		Grams	*Kilograms*	*Kilograms*	*Kilograms*
Quartz flour	50.0	21.4	1,645	76.9	153.8
Do	25.0	23.8	1,690	71.0	284.0
Do	10.0	21.2	590	27.8	278.3
Do	3.6	19.9	218	10.9	303.9
Medium sand	50.0	21.3	322	15.1	30.2
Do	40.0	21.9	281	12.8	32.1
Do	30.0	21.5	209	9.7	32.4
Do	20.0	20.4	100	4.9	24.5
Do	10.0	18.1	43	2.4	23.8

THE BINDING POWER OF DIFFERENT SOIL COLLOIDS

There were a few soil colloids available in the laboratory in sufficient quantity to make a few briquettes. These colloids were in the air-dry condition while the Sharkey colloid used in the above experiments was in the moist condition. The results of these tests are shown in Table IX. The five different colloids studied show rather wide variations in binding power. The different soil colloids show rather wide differences in adsorptive power, as well as in general character and appearance, so it was but reasonable to assume that there would be differences in binding power; however, the differences were not expected to be as great as these results indicate. Perhaps when these materials are available in larger quantities and in the moist condition, a more adequate explanation of the differences in binding power may be given.

TABLE IX.—*Showing the effect of the kind of colloid on the breaking strength of briquettes*

(a) 25 PER CENT COLLOID (AIR-DRY) AND QUARTZ FLOUR

Colloid from—	Weight of briquette	Average load per briquette	Load per gram of briquette	Load per gram of colloid
	Grams	*Kilograms*	*Kilograms*	*Kilograms*
Stockton clay adobe soil	24.0	1,726	72.0	288.0
Sharkey clay soil	21.9	1,015	46.4	185.5
Cecil clay loam subsoil	23.3	664	28.5	114.0

(b) 10 PER CENT COLLOID (AIR-DRY) AND FINE SAND

Norfolk fine sandy loam soil	18.8	61.2	3.3	32.5
Marshall silt loam soil	19.0	90.7	4.8	47.8
Sharkey clay soil	18.9	108.8	5.8	57.6

GENERAL DISCUSSION

Some of the factors influencing the binding power of soil colloids have been outlined above. It was first necessary to establish a suitable method for determining the binding power and to ascertain the conditions under which the results from this method could be relied upon. The method of determining the breaking strength of briquettes was adopted as most applicable, and when proper care was taken in making them, the results could be duplicated with sufficient accuracy.

In order to obtain duplicable and therefore comparable results the briquettes must be made up with (a) the critical amount of moisture present, that is, the moisture content when the briquettes with 2,000 pounds pressure applied assumed the smallest volume, (b) the same degree of dispersion of the colloid and a uniform distribution, (c) the same pressure per square inch (2,000 pounds) for the same length of time and (d) drying in such a manner as to prevent cracking of the briquettes. A variation of any of these factors might so affect the breaking strength of the briquettes that the values obtained would be very much in error. These four factors, which are involved in the formation of the briquettes and influence the values obtained, were standardized as nearly as possible. Working under the standardized conditions, the effect of the following factors on the binding power of the colloid was studied: (a) The amount of colloid present, (b) the kind of colloid, (c) the size of the mineral grains, (d) the grading of the sizes of the mineral grains, and (e) the surface area of the mineral grains. These factors are variable and interdependent so that great accuracy is not obtainable in determining them.

Tests on soils showed a general relation expressed in the formula $L = .42C^{1.34}$. An additional factor is involved, in the case of soil, in the determination of the amount of colloid in the soil. The variation in load is in direct proportion to the amount of colloid present, the constants in the equation changing with the character of the colloid and the mineral portion of the soil.

Tests on mixtures of colloid and sand show that with decreasing size of sand grains there is an increasing value for the binding power of the colloid. The increase in binding power is not directly proportional to the decrease in the average diameter of the sand grains. For example, in Table V the medium sand with 25 per cent colloid gives a load per gram of colloid of 17 kgm., but when very fine sand is used with a mean diameter one-fifth that of medium sand, the binding power is approximately twice as great. However, with the silt and clay sizes the increase in binding power is larger in proportion than the decrease in diameters. This indicates that the amount of surface has a very great influence. The calculation of surfaces as shown in Table VII shows that it is probable that a relation does exist, though the data for a complete verification of the relation are not available.

The kind of colloid is of great importance in determining the binding power of mixtures. Of those colloids tested, that from the Stockton clay adobe soil had the greatest binding power and that from the Cecil clay loam subsoil the least. The reason for this difference has not been determined, but it may be due to a difference in the chemical composition, in the size or aggregation of the colloidal particles, or to other factors.

This discussion gives some idea of the nature of the problem of determining the binding power of colloidal material in the soil. The complexity of the problem is pointed out and the influence of the various factors is recognized. While the factors and their influences have been partially determined, it yet remains to coordinate and fully evaluate them, so that more exact information of the character of a soil may be derived from the determination of the colloid content and one or more of these factors.

SUMMARY

1. A method is outlined for testing the binding power of soil colloids by determining the breaking strength of briquettes molded from soils under definitely established conditions.

2. It is shown that the factors influencing the breaking strength of a briquette are:

(*a*) The amount of moisture present at the time of molding.
(*b*) The treatment of the material before molding the briquette.
(*c*) The pressure applied.
(*d*) The manner of drying.

3. Some of the factors found to affect the binding power of soil colloids are:

(*a*) The amount of colloid present.
(*b*) The size and grading of the noncolloidal material.
(*c*) The kind of colloid.
(*d*) The dispersion of the colloid.

4. A general relation between the load per gram of soil L, and the amount of colloid in the soil in per cent C, has been deduced and given expression in the formula, $L=.42\ C^{1.24}$.

LITERATURE CITED

(1) ANDERSON, M. S., and others.
1922. ABSORPTION BY COLLOIDAL AND NONCOLLOIDAL SOIL CONSTITUENTS. U. S. Dept. Agr. Bul. 1122, 20 p.

(2) ARND, T.
1923. EIN NEUES VERFAHREN ZUR VERGLEICHENDEN BESTIMMUNG DER KOHÄRENZ MINERALISCHEN BÖDEN. Ztschr. Pflanzenernährung u. Düngung (A) 2: 130–149.

(3) ATTERBERG, A.
1912. DIE KONSISTENZ UND DIE BINDIGKEIT DER BÖDEN. Internat. Mitt. Bodenkunde 2: 149–189, illus.

(4) GILE, P. L., and others.
1924. ESTIMATION OF COLLOIDAL MATERIAL IN SOILS BY ADSORPTION. U. S. Dept. Agr. Bul. 1193, 41 p.

(5) JACKSON, F. H., Jr.
1916. METHODS FOR THE DETERMINATION OF THE PHYSICAL PROPERTIES OF ROAD-BUILDING ROCK. U. S. Dept. Agr. Bul. 347, 27 p., illus.

(6) MARQUIS, C.
1915. VERGLEICHENDE UNTERSUCHUNGEN ÜBER DIE METHODEN DER KOHÄRENZBESTIMMUNG. Internat. Mitt. Bodenkunde 5: 381–516, illus.

(7) MOORE, C. J., FRY, W. H., and MIDDLETON, H. E.
1921. METHODS FOR DETERMINING THE AMOUNT OF COLLOIDAL MATERIAL IN SOILS. Jour. Indus. and Engin. Chem. 13: 527–530, illus.

(8) PUCHNER, H.
1913. VERGLEICHENDE UNTERSUCHUNGEN ÜBER DIE KOHÄRESZENZ VERSCHIEDENER BODENARTEN. Internat. Mitt. Bodenkunde 3: 141–239, illus.

(9) ROBINSON, W. O.
1922. THE ABSORPTION OF WATER BY SOIL COLLOIDS. Jour. Phys. Chem. 26: 647–653.

THE GENETIC RELATION BETWEEN TRITICUM DICOCCUM DICOCCOIDES AND A SIMILAR MORPHOLOGICAL TYPE PRODUCED SYNTHETICALLY[1]

By H. H. Love, *Department of Plant Breeding, Cornell University Agricultural Experiment Station*, and W. T. Craig, *Agent*, Office of *Cereal Investigations, Bureau of Plant Industry, Department of Agriculture*

The synthetic production of a form similar to the wild emmer (*Triticum dicoccum dicoccoides* Kcke.) has been reported by the authors of this paper.[2] This synthetic form resulted from a cross between Early Red Chief, a variety of *Triticum vulgare* Vill., and one the writers received under the name Marouani, a variety of *Triticum durum* Desf. It is possible that the name Marouani was a misnomer, as Dr. C. R. Ball, on examining the sample, stated that it more nearly resembled the variety Peliss.

In the earlier paper it was noted that the chief difference between the true wild emmer and the synthetic form is the width of spikelets. The kernels of the synthetic type are broader than those of the true wild form as illustrated by the samples on hand. This causes the spikelets to be broader. The true wild emmer, however, is very variable and some forms are found that have very broad spikelets, even resembling the synthetic form.

One of the chief characteristics of the wild emmer is the fragility of the rachis. The articulation is such that at maturity the spikelets separate one from another very readily. The rachis segment which bears a spikelet remains attached to the base of the spikelet when the spike disarticulates, just as in ordinary emmer. Disarticulation occurs so easily that it is very difficult to obtain a head of the wild emmer intact. The synthetic form, Pl. 1, B, is very similar to this wild one, Pl. 1, A.

As the synthetic form, which was produced as a result of the cross, so nearly resembled the true wild emmer in all its visible characters, it seemed worth while to compare the two forms as to their genetic behavior. With this in mind the two have been crossed upon the same variety.

Two different kinds of crosses were made for this study. In one case a durum wheat, the Kubanka, was crossed with the two wild types, and in the other case both of them were crossed with Black Winter emmer. In the discussion of these results the synthetic type will be referred to as synthetic wild and the true wild emmer as true wild or simply as wild.

THE F_1 GENERATION

The F_1 plants resulting from the two crosses, where the true wild and synthetic wild were crossed with Kubanka, prove to be quite similar. The culms are solid or full of pith below the head. The spikes break up readily, indicating the domi-

[1] Received for publication March 29, 1924. Published as Paper No. 129, Department of Plant Breeding, Cornell University, Ithaca, New York.

[2] Love, H. H., and Craig, W. T. The synthetic production of wild wheat forms. Jour. Heredity 10: 51-64, illus. 1919.

Journal of Agricultural Research. Washington, D. C.

Vol. XXVIII, No. 6
May 10, 1924
Key No. G-413

96036—24†——2

nance of the fragile rachis. On first examination it seemed that those heads coming from the cross in which the true wild was used were more fragile, but after thorough drying there seemed to be no difference. The F_1 heads in both crosses have a flattened appearance similar to those of the wild type and the rachis is heavily pubescent, as is the case in the wild forms. The awns resemble those of the wild parents and the kernels have the red color of the wild forms. In shape the kernels are longer than those of Kubanka, but are somewhat broader than those of the wild forms.

The F_1 plants which resulted from crossing the two wild types with Black Winter emmer were alike in each case. So far as the characters mentioned above are concerned, these F_1 plants were similar to those from the Kubanka crosses with the exception that the glumes of the hybrids with Black Winter emmer were black or grayish-black. The color is slightly lighter than that of the Black Winter emmer.

THE F_2 GENERATION

When the second generation was grown there was considerable diversity of form and of degree of fragility of the rachis. Some of the characters will be discussed separately.

The first character to be considered is the fragility of the rachis. Owing to the nature of this character, it is very difficult to classify. This is especially true if the material has not dried thoroughly. Some of the rachises are brittle only toward the tip of the head while others may be fragile the entire length of the head, except for a few spikelets at the base. In the wild emmer usually two or three spikelets at the base of the head are held rather firmly.

Various tests were used in order to classify this material, such as natural shattering, readiness of breaking when the spike was shaken, or when the spikes were subjected to some stress, and so on. Finally, the classification was made by means of the above tests plus an examination of the end of the rachis segment under a lens. The rachis of the true wild emmer disarticulates in such a way that the end of the rachis segment is round and perfectly smooth. This test was then applied and all plants that showed disarticulation leaving the rachis segments with smooth round ends were classed as fragile. If the end of the rachis segment was broken or torn the plants were classed as tenacious. The results of segregation for this character in the second generation plants are given in Table I.

TABLE I.—*Segregation of the second generation of crosses of Kubanka durum wheat and Black Winter emmer with the wild and the synthetic wild emmer for character of rachis, shown by manner of disarticulation*

Cross	Number of plants having—				Deviation	Probable error	Deviation divided by probable error
	Fragile rachis		Tenacious rachis				
	Observed	Calculated	Observed	Calculated			
Kubanka X true wild	134	133.5	44	44.5	0.50	3.90	0.13
Kubanka X synthetic wild	147	150.0	53	50.0	3.00	4.13	0.73
Black Winter emmer X true wild	216	219.38	18	14.62	3.38	2.50	1.35
Black Winter emmer X synthetic wild	221	227.8	22	15.20	6.80	2.55	2.67

From these results it is very evident that, so far as this character is concerned, the segregation for fragile and for tenacious rachis gives about the same ratio whether the wild or synthetic type is used as the parent. It seems evident that

in the crosses with Kubanka, the segregation follows the simple 3 : 1 ratio. The deviation from the expected numbers is very small compared with the probable errors. This ratio was established further by an examination of some F_2 material.

In the case of the crosses with Black Winter emmer a different condition exists. Here the segregation suggests a 15 : 1 ratio. The deviation from the expected numbers is within the limit of three times the probable error in each case. Such behavior may be expected from emmer as it has a different method of disarticulation from that of Kubanka. The emmer spike does not break up readily but when the rachis disarticulates the spikelet carries the rachis segment which bore it. This fact would lead one to expect a behavior in inheritance different from that in the case of the Kubanka wheat.

These results show very plainly that, so far as these two crosses are concerned, the true wild and the synthetic wild are very similar in their behavior with respect to the inheritance of the character of the rachis.

The different types of heads obtained from these crosses are shown in Plates 2 to 8, inclusive.

In Plate 2 are shown two heads resembling those of Black Winter emmer. Head A was obtained from crossing Black Winter emmer with the true wild, and head B from crossing it with the synthetic wild.

The heads shown in Plate 3 also are from the crosses with Black Winter emmer. Heads A and C are from the cross with the true wild and heads B and D from that with the synthetic wild. These illustrations show the great similarity between the heads from the two different crosses. Heads A and B have a tenacious rachis and heads C and D a fragile rachis. These heads all have black glumes.

The heads in Plate 4 also are from the crosses between Black Winter emmer and the two wild emmers. These heads have brown glumes and are selected to show the tenacious and the fragile rachis from the two crosses. Heads A and C are from the cross in which the wild was used as a parent and heads B and D are from the cross where the synthetic wild was used.

The heads in Plate 5 also are from the crosses between Black Winter emmer and the two wild forms. Heads A and B are from the cross with the true wild and heads C and D from the other cross. All have white glumes. They again show the close resemblance between the heads from the two crosses.

The heads from the crosses between Kubanka durum and the two types of wild emmer are shown in Plates 6, 7, and 8. Heads A and C of each plate are from the cross with the true wild and heads B and D of each plate are from the cross with the synthetic wild. The heads in Plate 6 resemble those of emmer to some extent. The heads in Plate 7 are more like those of durum, and the heads in Plate 8 are similar to those of spelt except that the articulation is more like that of emmer. In general, it is clear that heads obtained from the two types of crosses are very similar and indicate that the synthetic wild carries the same factors for head type as does the true wild.

These crosses did not show any segregation for color of kernel in the series where Black Winter emmer was used. This indicated that the constitution of the two types of wild was the same as that of Black Winter emmer. In the crosses where Kubanka was used, however, segregation did occur. (See Table II.)

TABLE II.—*Segregation in F_2 for color of kernel in crosses between Kubanka durum and the two wild emmers*

Cross	Numbers of plants and kernel color				Deviation	Probable error	Deviation divided by probable error.
	Observed		Expected				
	Red	White	Red	White			
Kubanka X true wild	164	14	166.9	11.1	2.9	2.18	1.33
Kubanka X synthetic wild	185	15	187.5	12.5	2.5	2.31	1.08

TABLE III.—*Segregation in F_2 for color of glumes in crosses between Black Winter emmer and the two wild emmers*

Cross	Numbers of plants having color of glumes			
		Black	Brown	White
Black Winter emmer X true wild	Observed	181	43	10
	Expected	175.2	43.8	14.6
Black Winter emmer X synthetic wild	Observed	173	53	17
	Expected	182.4	45.6	15.2

These results indicate a 15:1 ratio and the deviations are not very large. In fact, when they are compared with their probable errors they are not significantly different from what one would expect. The red color of the true wild and the synthetic wild must, therefore, depend upon two factors.

From another cross where the true wild was used a segregation of 15 red:1 white was obtained. It is rather interesting that this synthetic wild emmer should have the same genetic constitution for color of kernel as the true wild.

The crosses between Kubanka and the wild emmers did not show any segregation for color of glumes, as both parental forms had white or yellowish-white glumes. In the case of the Black Winter emmer crosses, however, there was segregation, as would be expected, with contrasted glume colors. (See Table III.)

The results show that the segregation follows a 12:3:1 ratio. That is, the Black Winter emmer carries both the factor for black or purple glume color and the one for brown color. While the observed and expected numbers show some deviation, they are in fair agreement, considering the numbers of plants. For the cross where the true wild emmer was used P equals 0.450, and for the one where the synthetic wild was used P equals 0.392.

The distribution and closeness of fit show very clearly that the two types of wild emmer react in a very similar way when crossed upon the Black Winter emmer. This fact is further emphasized when the pubescence of the glume is considered. The pubescence of the glume of the Black Winter emmer is linked with the black or purple color of the glume. The cross with the true wild gives 181 black pubescent and 53 nonblack glabrous, while the cross with the synthetic wild gives 173 black pubescent and 70 nonblack glabrous. These numbers deviate somewhat from the expected 3:1 ratio, yet if the numbers of plants were larger no doubt the agreement would be closer. Regarding other characters, such as the number of spelt or emmer types occurring in the different crosses, the data show that the results are about the same no matter whether the true wild or synthetic wild was used as a parent.

So far as these studies have been conducted it is evident that the synthetic wild emmer, which occurred as a result of crossing a variety of *Triticum vulgare* and a variety of *Triticum durum*, is the same as, or certainly genetically very similar to, the true wild emmer found in Palestine.[3] The earlier paper mentioned above[4] shows that, so far as the visible characters are concerned, the two forms are alike, and it is a very interesting fact that genetically the same is true. These two forms are now being compared in other ways. The evidence seems to indicate that the synthetic form will repeat in every way the behavior of the true wild form.

Such evidence, however, does not yet answer the question as to whether this wild emmer, *Triticum dicoccum dicoccoides*, is the progenitor of all other types. Argument may be presented that it is the progenitor or that it is only a contemporary form. One fact has been established, namely, that through hybridization a form similar morphologically and genetically to the true wild type has been found. That this is no mere accident is supported by the fact that from still other crosses other synthetic forms of wild emmer have been obtained.

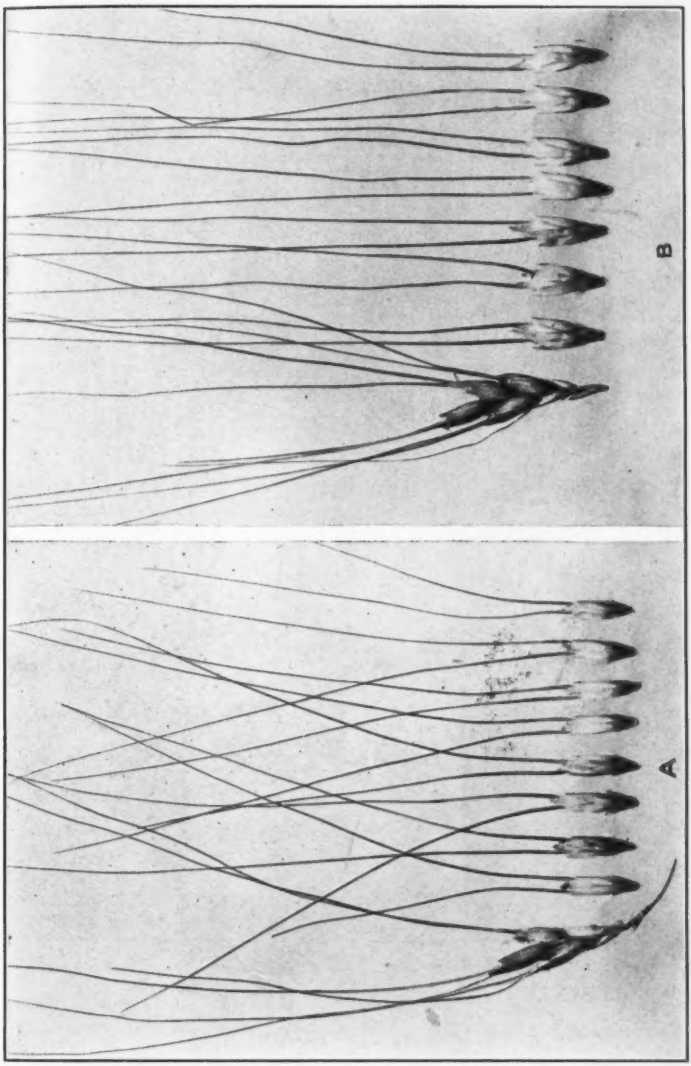
[3] AARONSONN, A. AGRICULTURAL AND BOTANICAL EXPLORATIONS IN PALESTINE. U. S. Dept. Agr. Bur. Plant Indus. Bul. 180, 64 p., illus. 1910.

[4] LOVE, H. H., and CRAIG, W. T. THE SYNTHETIC PRODUCTION OF WILD WHEAT FORMS. Jour. Heredity 10: 51-64, illus. 1919.

PLATE 1

Basal portion of spike and individual spikelets of the true wild (A) and synthetic wild (B) emmers.

Comparison of Natura and Synthetic Wild Emmers PLATE I



Comparison of Natural and Synthetic Wild Emmers

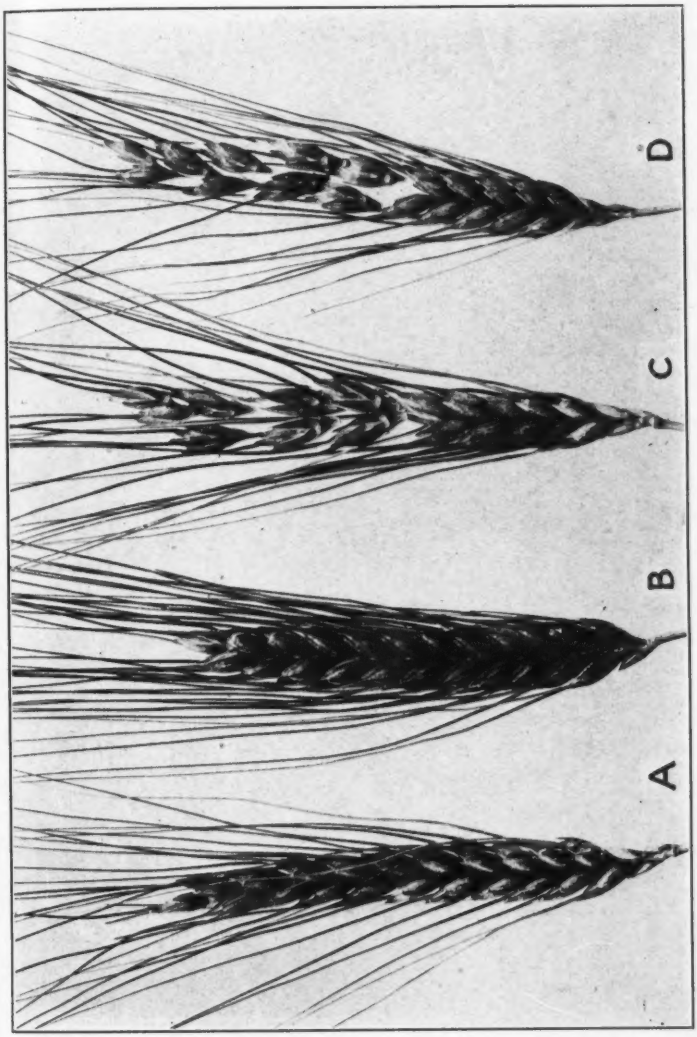
PLATE 2

PLATE 2

Spikes of the F_1 generation, resembling those of Black Winter emmer, resulting from crossing Black Winter emmer with the true wild and synthetic wild emmers.

PLATE 3

Black-glumed spikes of the F_2 of the crosses between Black Winter emmer and the two wild emmers. Heads A and C from the cross between the true wild and heads B and D from the cross with the synthetic wild.


A
B
C
D

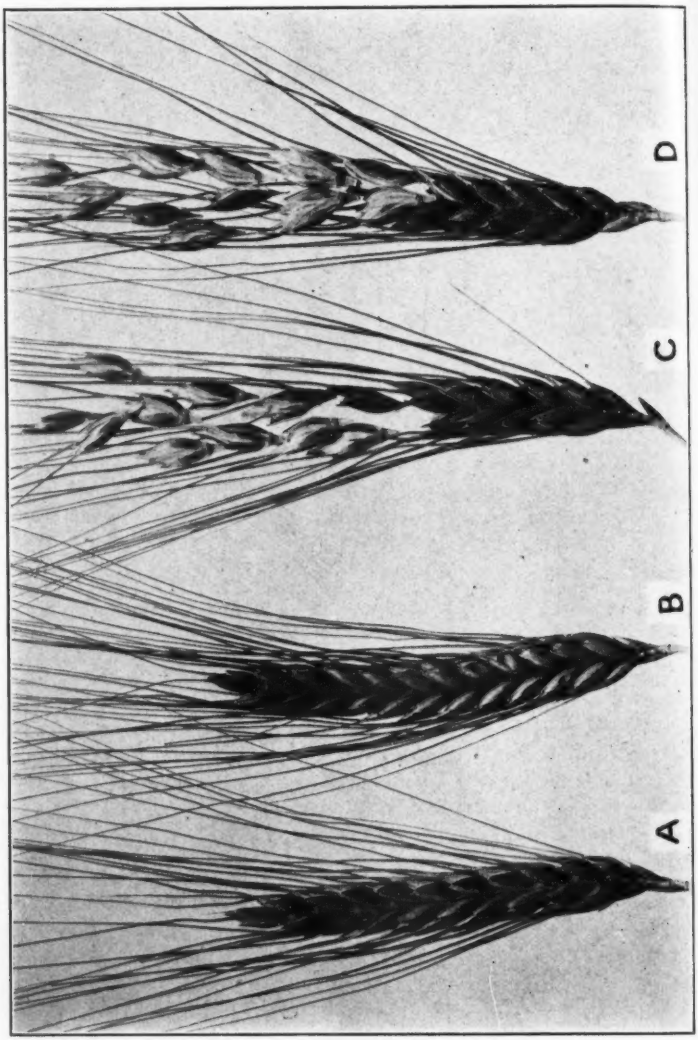
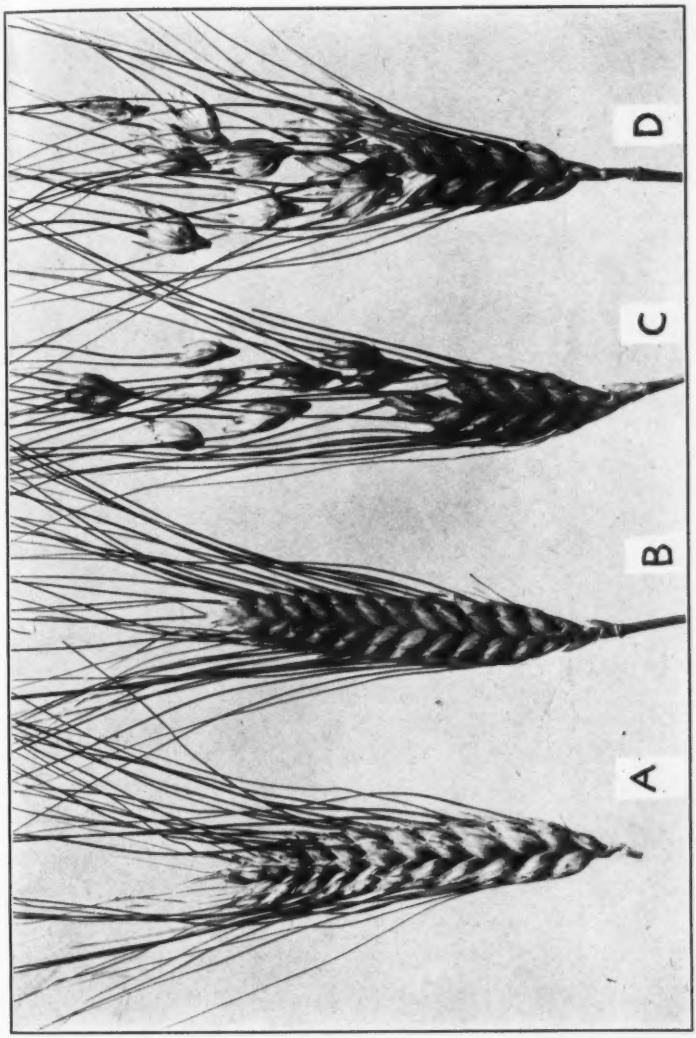

A
B
C
D

PLATE 4

Brown-glumed spikes of the F_2 of the crosses between Black Winter emmer and the two wild emmers. Heads A and C from the cross between the true wild and heads B and D from the cross with the synthetic wild.

PLATE 5

White-glumed spikes of the F_2 of the crosses between Black Winter emmer and the two wild emmers. Heads A and C from the cross between the true wild and heads B and D from the cross with the synthetic wild.


D
C
B
A

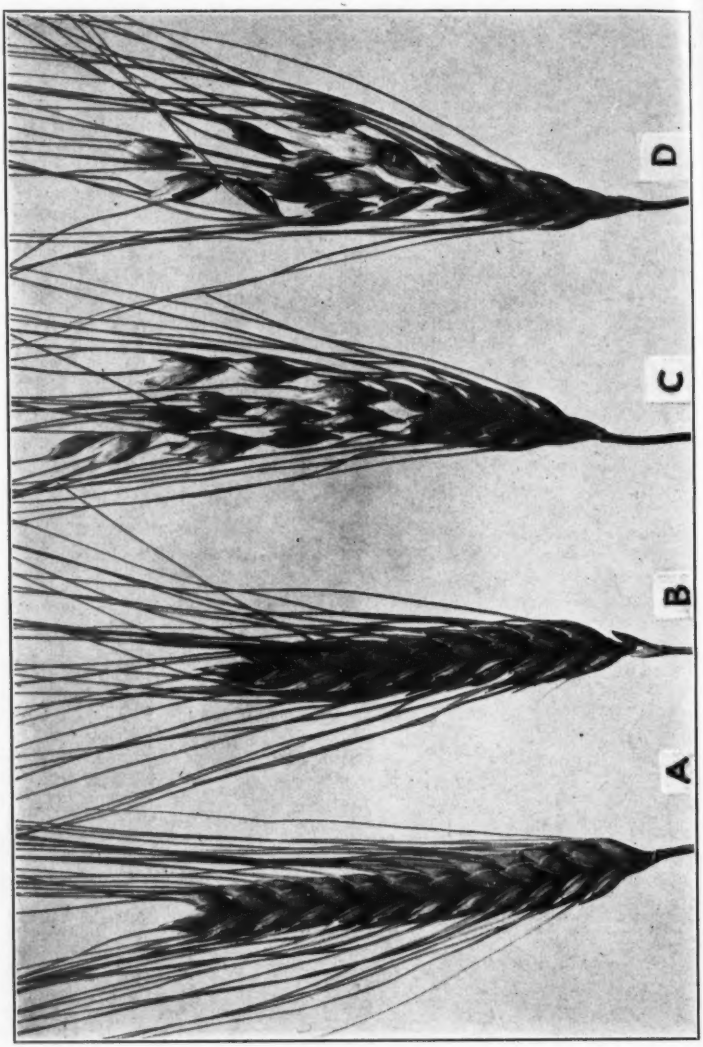
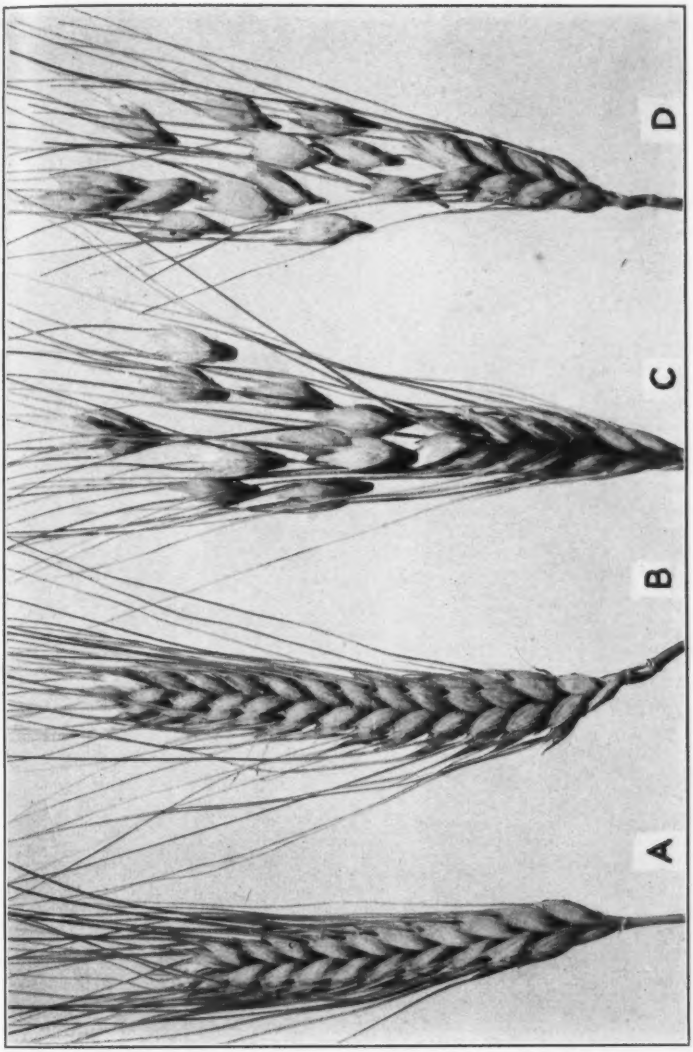

A
B
C
D

PLATE 6

Spikes, somewhat resembling those of emmer, from the F_2 generation of the cross between Kubanka durum and the two kinds of wild emmer. Heads A and C from the cross between the true wild and heads B and D from the cross with the synthetic wild.

PLATE 7

White-glumed spikes, resembling those of durum wheat, from the cross between Kubanka durum and the two kinds of wild emmer. Heads A and C from the cross between the true wild and heads B and D from the cross with the synthetic wild.


A
B
C
D

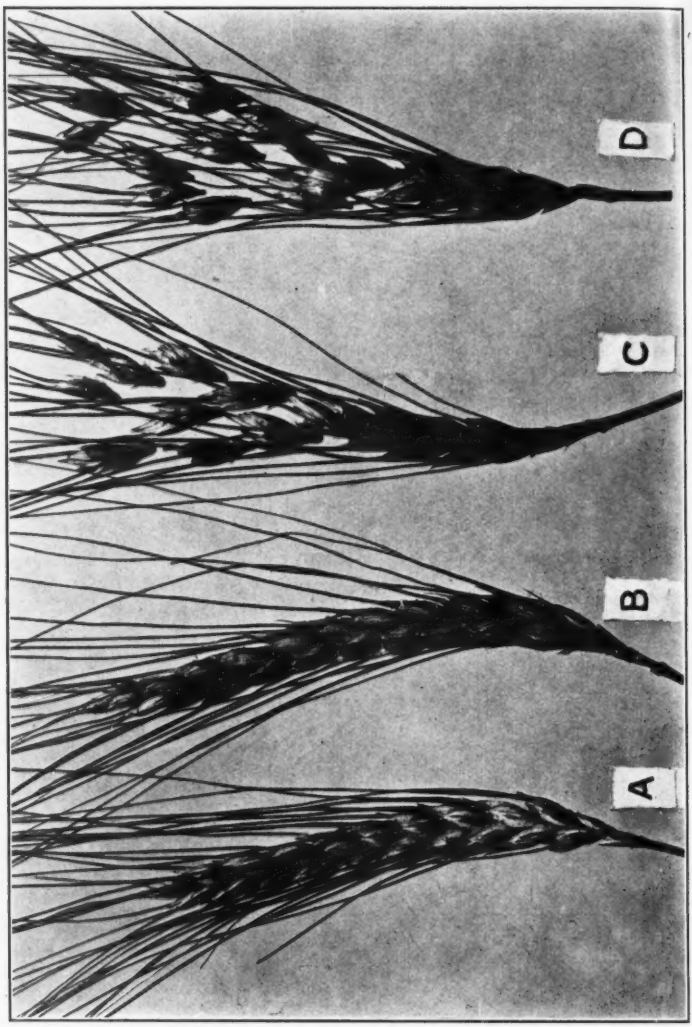

D
C
B
A

PLATE 8

Spelt-like spikes of the F_2 from the cross between Kubanka durum and the two kinds of wild emmer. Heads A and C from the cross between the true wild and heads B and D from the cross with the synthetic wild.

BUD SELECTION AS RELATED TO QUALITY OF CROP IN THE WASHINGTON NAVEL ORANGE[1]

By A. D. SHAMEL, *Physiologist in Charge*, C. S. POMEROY, *Pomologist*, and R. E. CARYL, *Assistant Pomologist, Fruit Improvement Investigations, Office of Horticultural Investigations, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

The occurrence of bud variations in citrus trees and the relation of some of them to the origin and development of a number of strikingly diverse strains of the commercial citrus varieties grown in California have been presented in earlier publications.[2] Evidence that quantity of production may be perpetuated through bud propagation of limb variations of the Washington Navel orange has recently been published.[3] Many marked differences in quality of fruit, apparently resulting from bud variation, have been under observation and several are now being studied in progeny plantings. A variation of fruit quality, directly traceable to bud variations in trees of both the Washington and Thomson strains of the Washington Navel orange, is herein described.

FACTORS DETERMINING THE QUALITY OF CROP

The term "quality of crop" is here used to define the market grade of the fruit as affected by its physical characteristics. The size, color, shape, texture, and blemishes of the fruit determine its commercial grade in large measure. The thickness of the rind, amount and flavor of the juice, character of the rag, and the number of seeds are also important physical characteristics of the fruits from the commercial standpoint. Fruits of medium size for the variety are usually the most valuable, being most desired by the consumer and most profitable for the producer. A reddish-orange color is most desirable, while a pale or yellowish color usually lowers the merchandising value of the fruit. In connection with their packing and marketing, uniformity of shape within the variety is an important character. Corrugated, rough fruits or those having an uneven surface are usually assorted into lower grades, a skin of uniformly smooth texture being most desirable. Blemishes most commonly found are scars, scratches, insect injuries, diseased areas, and spots which are apparently due to physiological weaknesses of the rinds.

---

[1] Received for publication January 22, 1924.

[2] The following department bulletins summarize the results of the studies of bud variations in the Washington Navel and Valencia orange, Marsh grapefruit, and Eureka and Lisbon lemon varieties, respectively:

SHAMEL, A. D., SCOTT, L. B., and POMEROY, C. S. CITRUS-FRUIT IMPROVEMENT: A STUDY OF BUD VARIATION IN THE WASHINGTON NAVEL ORANGE. U. S. Dept. Agr. Bul. 623, 146 p., illus. 1918.

——— ——— ——— CITRUS-FRUIT IMPROVEMENT: A STUDY OF BUD VARIATION IN THE VALENCIA ORANGE. U. S. Dept. Agr. Bul. 624, 120 p., illus. 1918.

——— ——— ——— CITRUS-FRUIT IMPROVEMENT: A STUDY OF BUD VARIATION IN THE MARSH GRAPEFRUIT. U. S. Dept. Agr. Bul. 697, 112 p., illus. 1918.

——— ——— ——— CITRUS-FRUIT IMPROVEMENT: A STUDY OF BUD VARIATION IN THE EUREKA LEMON U. S. Dept. Agr. Bul. 813, 88 p., illus. 1920.

——— ——— ——— CITRUS-FRUIT IMPROVEMENT: A STUDY OF BUD VARIATION IN THE LISBON LEMON U. S. Dept. Agr. Bul. 815, 70 p., illus. 1920.

[3] SHAMEL, A. D., POMEROY, C. S., and CARYL, R. E. BUD SELECTION AS RELATED TO QUANTITY PRODUCTION IN THE WASHINGTON NAVEL ORANGE. Jour. Agr. Research 26: 319-322, illus. 1923.

Journal of Agricultural Research, Washington, D. C.

Vol. XXVIII, No. 6
May 10, 1924
Key No. G-420

PROGENY TESTS OF BUD VARIATIONS

The individual-tree performance-record studies of many trees possessing marked limb variations which have been described in earlier reports suggest the probability that the established trees of diverse strains occurring in the performance-record plats and similar ones observed in commercial orchards were unintentionally propagated from limb variations.

In order to secure definite evidence upon the perpetuation of diverse strains with especial reference to quality of fruit, experimental propagations were begun in 1915 using bud wood secured from limb variations which resembled in fruit and foliage characteristics some of the trees of several of the strains under investigation. These propagations were made on selected sour orange seedlings in a commercial nursery in cooperation with the Citrus Experiment Station of the University of California and some of the resulting progenies were planted on the grounds of the Citrus Experiment Station at Riverside, Calif., in July, 1917. The land on which these progeny plantings are located had previously been planted only to winter grain crops which had been grown without irrigation or fertilization. Little or no fertilizer has been used in this experimental progeny orchard other than that secured from the legume cover crop growth which has been plowed under once or twice each year and no pruning of the trees has been done thus far.

Each progeny was planted so that the trees stand 10 feet apart in the same row, this close planting providing for many more trees in the available area than could otherwise have been grown. The usual cultivation and irrigation methods have been practiced and orchard heaters have been provided for frost protection during periods of low temperatures. The performance record of each tree has been secured every year since they came into bearing, particular attention being given to recording the number and quality of variants from the normal type of fruit.

BROWN-SPOTTED AND EARLY RIPENING LIMB VARIATIONS

The parent tree in which the first limb variation of this type was discovered is a tree of the Thomson strain of the Washington Navel orange variety on the Victoria ranch of the National Orange Co., at Riverside, Calif., which came to the attention of the senior writers in 1915 when it was 12 years old. The rootstock used in the propagation of the parent tree was sweet seedling orange. The size of the tree and the appearance of the foliage indicated a normal and healthy condition of growth.

The fruits borne by the parent tree are quite variable, the majority being classed as typical of the Thomson strain while some of them very closely resemble typical fruits of the Washington strain. The Thomson fruits are distinguished by very smooth and comparatively thin rinds having a bright reddish-orange color. The flesh is coarse and the fruits usually lack juice to such a degree as to render them very much less desirable than are those of the Washington strain. The fruits of the Washington strain have somewhat coarser and thicker orange-colored rinds but have very tender flesh with an abundance of juice, making them very desirable.

One limb of this parent tree produces very early ripening fruits of small size, distinctly flattened, pale yellow in color with occasional reddish stripes and marked with sunken brown spots of irregular shapes and sizes. The spotted oranges usually have only a small or rudimentary navel and frequently the navel opening is entirely closed. The rinds of the fruits are usually very thick and the flesh coarse and tough. The fruits ordinarily have but little juice which is normally lacking in both acid and sugar, making them very undesirable. The fruits mature and most of them drop from the tree during November and De-

cember and after the oranges drop the brown spots increase in number and size. Plate 1, A, shows typical fruits from this limb variation and Plate 1, B, shows typical Thomson fruits from a normal limb on the same tree. It was first thought that these spots were due to fumigation burns, and when this theory was proven to be incorrect it was suggested that they might have been caused by hail injuries. The fact that the fruits on adjacent trees and those on the parent tree other than the ones borne by the limb variation were free from these spots indicated that they were not caused by hail. This conclusion has been confirmed by the fact that in each successive season the fruits on the limb variation have shown similar spots, while the fruits on the other branches have remained normal in this respect, and during several of these seasons no hail fell in the vicinity.

It was thought for a time that the spotted condition of the fruit might be due to a fungus disease, but careful pathological studies by two independent investigators failed to reveal any evidence of disease. Subsequent observations and studies of the spotted fruits have led to the conclusion that the tissue of the rinds of the fruits is inherently weak and breaks down rather easily as the oranges approach maturity. The spots have a similar appearance to those which develop on citrus fruits where the oil cells have been broken and the free oil becomes spread over the adjacent areas of the rinds.

The parent tree on which the second limb variation of this kind was found is a tree of the Washington strain of the Washington Navel orange variety, which had been propagated on sweet-seedling orange rootstock and planted in 1912. This parent tree is located in the orchard of the Lemona Heights ranch, which is about 3 miles distant from the Victoria orchard where the other tree showing this type of variation was found.

The limb variation in the tree of the Washington strain was found to produce fruits which were very similar to those borne by the limb variation in the tree of the Thomson strain described. The fruits are smaller than those borne by the normal branches of the tree, more flattened in shape, and have a yellowish color with occasional red stripes or sections. The rinds are thicker and coarser in texture than is the case with the normal fruits on this tree. The fruits mature much earlier than those on the normal branches and drop easily, most of them falling to the ground during the months of November and December. The fruits develop small, irregular sunken brown spots on the surface of the rinds very similar in appearance to those found on the limb variation described above. None of these brown spots has been found on the reddish-colored sections which occur on some of the fruits.

PROGENY PROPAGATION

Buds secured from each of the limb variations of the two parent trees described above were propagated on sour orange seedlings in the spring of 1915. The resulting trees were planted on July 3, 1917, in the orchard of the Citrus Experiment Station of the University of California, only two of each progeny being planted owing to the limited available space. In the case of the Thomson strain variation, the progeny test consists of two trees grown from buds secured from the spotted-fruit limb variation and two trees grown from buds taken from a normal Thomson limb.

The progeny trees have made a healthy and normal growth and came into production during the 1920–21 season when they were 3 years old. All four of the progeny trees from the two spotted-fruit limb variations are smaller in size and have a more compact habit of growth than normal or typical trees of the strains. Plate 2 shows the comparative size and habit of growth of a progeny tree propagated from the spotted-fruit limb variation in the parent Thomson tree and a progeny tree from the normal portion of the same parent tree.

96036—24†——3

PROGENY PERFORMANCE RECORDS

The performance records of the progeny trees of the two spotted-fruit limb variations of the Thomson and Washington strains and of the normal part of the parent tree of the Thomson strain are shown in Tables I, II, and III.

TABLE I.—*Number of oranges produced by the two progeny trees which were propagated from the spotted-fruit limb variation in a tree of the Thomson strain*

Tree No.	1920–21	1921–22	1922–23	1923–24	Total
1	0	1	59	155	215
2	25	8	92	282	407
Total	25	9	151	437	622

TABLE II.—*Number of oranges produced by the comparative progeny trees propagated from a normal limb of the parent tree of the Thomson strain*

Tree No.	1920–21	1921–22	1922–23	1923–24	Total
1	10	95	92	242	439
2	7	107	96	370	580
Total	17	202	188	612	1,019

It will be noted that the progeny trees of the spotted-fruit limb variations of the Thomson strain have been less productive thus far than the comparative progeny trees grown from buds secured from a normal branch of the parent tree.

The fruits borne by the two progeny trees propagated from the limb variation in the tree of the Thomson strain have all developed the characteristic brown spots that were shown by the fruits on the parent limb, except four fruits in the crop of 1923–24 on tree No. 1, which had normal Washington texture with ribbed surfaces. The progeny trees propagated from the normal part of the same parent tree have produced only normal Thomson fruits. Typical fruits from one of the progeny trees propagated from the spotted-fruit limb variation are shown in Plate 3, A, and typical fruits from one of the progeny trees propagated from a normal limb of the same parent tree are shown in Plate 3, B.

The progeny trees propagated from the limb variation of the tree of the Washington strain have produced a few normal unspotted fruits and a few brown-spotted yellow fruits with unspotted reddish orange sections in addition to the spotted fruits, as shown in Plate 4, A. These types are similar to those which have been occasionally found on the parent limb. Typical spotted fruits from the second progeny tree are shown in Plate 4, B. The number of fruits of these various types produced by the progeny trees is shown in Table III.

TABLE III.—*Types of fruit borne on the two progeny trees propagated from the spotted-fruit limb variation in a tree of the Washington strain*

Type of variations	Number of fruits produced									
	1920–21		1921–22		1922–23		1923–24		Totals	
	Tree No. 1	Tree No. 2	Tree No. 1	Tree No. 2	Tree No. 1	Tree No. 2	Tree No. 1	Tree No. 2	Tree No. 1	Tree No. 2
Brown-spotted	11	44	7	17	45	50	134	268	197	379
Brown-spotted with reddish-orange sections	0	0	4	17	1	6	20	20	25	43
Normal Washington	5	0	7	2	4	7	25	2	41	11
Washington texture and ribbed	1	0	2	0	2	0	11	1	16	1
Total fruits	17	44	20	36	52	63	190	291	279	434

The spotted oranges borne by the progeny trees ripen and drop prematurely and after the fruits drop the sunken brown spots increase in number and size, frequently covering a large portion of the surface of the fruits, in the same manner as is the case with the fruits borne by the parent limbs.

CONCLUSION

The results of these progeny tests indicate that the brown-spotted and early-ripening characters of the fruits which have been described in the foregoing pages may occur as bud variations and that such limb variations may be perpetuated through bud propagation.

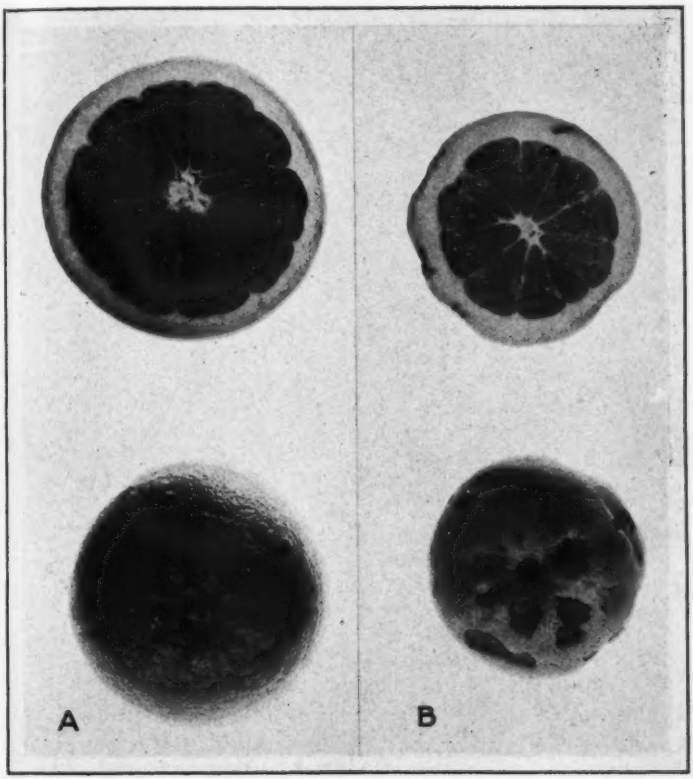
These studies suggest the probability that undesirable trees in established citrus orchards frequently may be due to unintentional propagations from similar limb variations.

These progeny tests emphasize the fundamental importance of careful bud selection in the commercial propagation of citrus trees.

PLATE 1

A.—Typical brown-spotted, yellow oranges borne by a limb variation in the tree of the Thomson strain of the Washington Navel orange variety. Victoria ranch, Riverside, Calif. Photographed January 8, 1923. Negative 2160.

B.—Typical Thomson oranges borne by the normal limbs of the Thomson Navel orange tree. Victoria ranch, Riverside, Calif. Photographed January 8, 1923. Negative 2159.


A
B

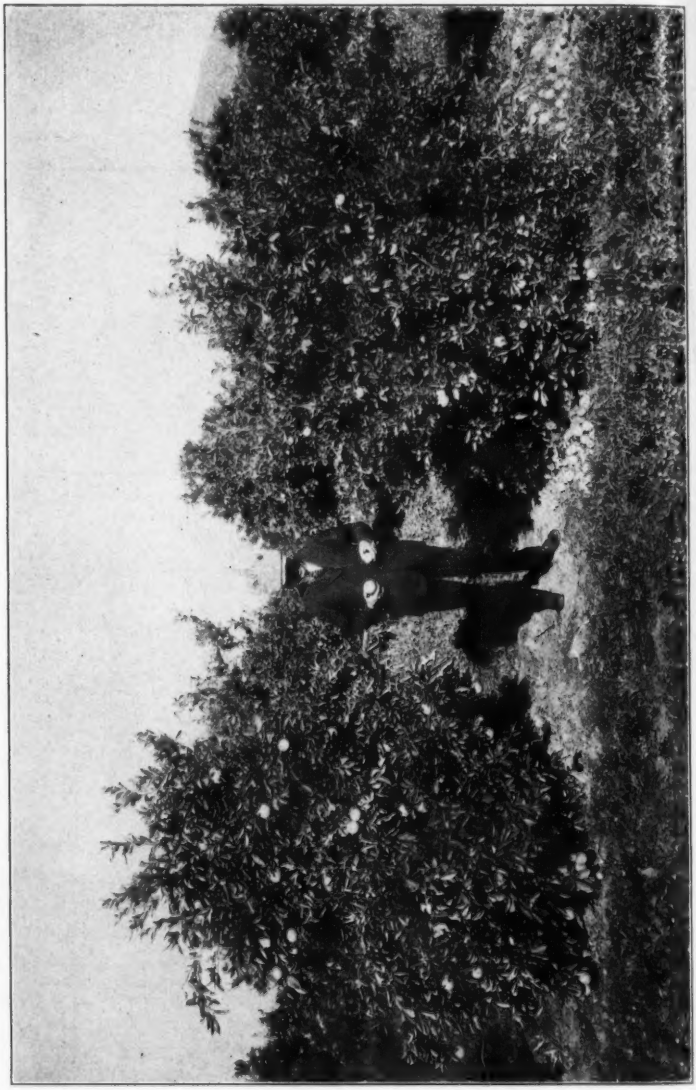


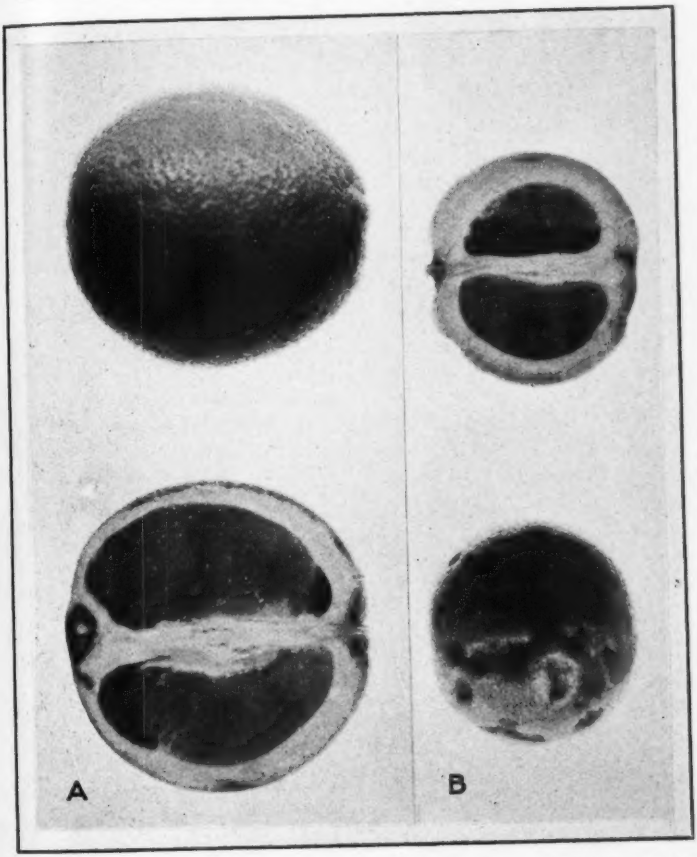
PLATE 2

Progeny orange trees propagated from (right) the limb variation which bears brown-spotted fruits and from (left) a normal branch of the same parent Thomson Navel tree. University of California Citrus Experiment Station, Riverside, Calif. Photographed January 5, 1924. Negative 2194.

PLATE 3

A.—Typical brown-spotted yellow oranges borne by one of the progeny trees which was propagated from the limb variation which bears similar fruits in the tree of the Thomson strain of the Washington Navel orange. Both the trees of this progeny produce fruit like that shown above. University of California Citrus Experiment Station, Riverside, Calif. Photographed January 5, 1923. Negative 2132.

B.—Typical Thomson Navel oranges borne by one of the progeny trees which was propagated from a normal limb of the tree of the Thomson strain of the Washington Navel orange. The other tree in this progeny produces fruit of this same character. University of California Citrus Experiment Station, Riverside, Calif. Photographed January 5, 1923. Negative 2136.


A
B

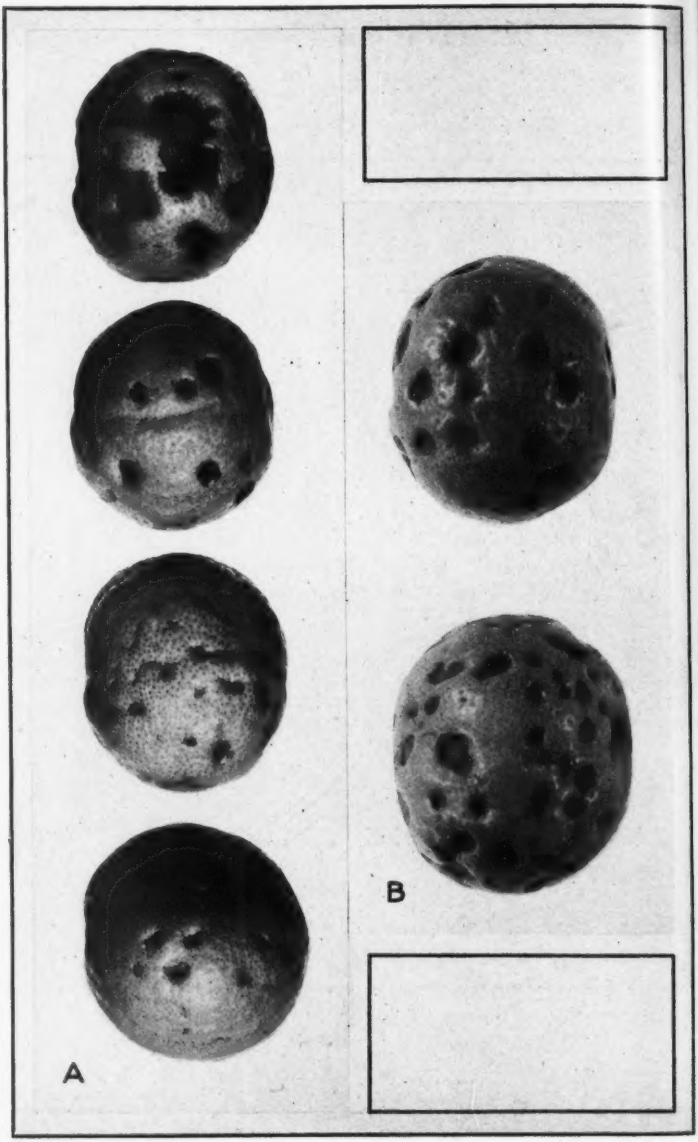

A
B

PLATE 4

A.—Typical oranges produced by progeny tree No. 1, which was propagated from a limb variation which bears brown-spotted, yellow fruits in a tree of the Washington strain of the Washington Navel orange variety. Note the normal Washington fruit, the ribbed Washington fruit, fruits showing normal and ribbed Washington sections, and fruits showing the spotted condition in varying degrees. University of California Citrus Experiment Station, Riverside, Calif. Photographed January 5, 1924. Negative 2197.

B.—Typical brown-spotted yellow-colored oranges produced by progeny tree No. 2, which was propagated from the limb variation which bears brown-spotted fruits in a tree of the Washington strain of the Washington Navel orange variety. University of California Citrus Experiment Station, Riverside, Calif. Photographed December 17, 1923. Negative 2188.

THE DETERMINATION OF NITRATE AND AMMONIA IN NITROGENOUS MATERIALS[1]

By O. M. SHEDD, *Research Chemist, Kentucky Agricultural Experiment Station*[2]

INTRODUCTION

The estimation of nitrate and of ammonia in the presence of other soluble and easily decomposable nitrogenous compounds of organic origin is difficult. They are determined by colorimetric methods or by procedures involving either gravimetric or volumetric features, and if present in large amounts the latter have proved more reliable. The present work consists mainly of a comparison of a recent Devarda alloy method devised by Strowd (*6*)[3] for the determination of nitrates in plants and a slight modification of same whereby the control determination in the modified method was used to estimate the ammonical nitrogen present in some samples. The latter has been made an incidental study, however, and the results obtained in this connection are included here, inasmuch as the procedure described may possibly be used for this determination on other materials.

HISTORICAL REVIEW

It has been recognized from the earliest work on the subject that the estimation of nitrate in the presence of organic nitrogenous materials presents difficulties. Some colorimetric methods such as those devised by Caron (*2*), and particularly the modified phenolsulphonic acid procedure of Chamot and coworkers (*3*), have been found very satisfactory under certain conditions, provided the amount of nitrate present is small and a clear solution of it can be obtained. Other methods are more satisfactory when larger amounts of nitrate are present and among them some form of reduction process is generally used. Gasometric methods involving either the Schlösing method or modifications (*7, p. 456*) have found favor with some workers. The "nitron" method (*7, p. 451*) has also been found to be applicable in certain cases. If no separation of the nitrate is to be made, but it is desired to include it with other forms of nitrogen that may be present, one of the improved salicyl-sulphonic acid procedures may be used (*1, p. 7, 8*).

The methods that are most popular at present, however, involve some reduction process whereby the nitrate is reduced to ammonia by nascent hydrogen generated by the action of an alkali or acid on one or more metals. The ammonia is afterwards distilled into an excess of standard acid and titrated. Briefly mentioned, they are the ferrous sulphate-zinc-soda method, which is carried on in an alkaline solution and recommended for nitrogen in commercial nitrate; the zinc-iron method, also carried on in an alkaline solution; and the Ulsch-Street, or reduced iron method, in which the nitrate is reduced in an acid solution by the action of sulphuric acid on reduced iron. The above three methods have been adopted as official in fertilizer work by the Association of Official Agricultural Chemists (*1, p. 10*). Some other methods which have been used are reduction with aluminum in an alkaline solution according to Pozzi-Escot (*5*) and

[1] Received for publication April 1, 1924. Published by permission of the director of the Kentucky Agricultural Experiment Station.

[2] The writer desires to thank Dr. A. M. Peter, head of the Department of Chemistry, for helpful criticisms during this investigation and in the preparation of the manuscript.

[3] Reference is made by number (italic) to literature cited, p. 538, 539.

reduction with Devarda's alloy (50 parts copper, 45 parts aluminum, and 5 parts zinc), conducted in an alkaline solution and now known as the Devarda alloy method (*7, p. 456*). The last procedure with some modifications has been found to be very satisfactory by many workers, and it is now becoming more generally used. Further work, together with minor improvements, will probably extend its application.

EXPERIMENTAL WORK

The writer, having occasion to make a large number of nitrate determinations in cured tobacco, desired to find a satisfactory method for this purpose. Previous experience had shown that if a distillation is made of tobacco with water and magnesium oxid according to the official procedure for ammoniacal nitrogen in fertilizers (*1, p. 10*), ammonia is slowly evolved from the nitrogenous compounds present and comes over long after a sufficient volume of distillate has been collected to account for any ammonia compound that may have been present originally.

The writer was most impressed with Strowd's method for the determination of nitrites and nitrates in plants (*6*) which is a modification of the Devarda alloy method. Strowd found that his procedure was more satisfactory for this purpose than any other method tried. One good point in this procedure is that it includes a control determination with each sample, without the Devarda alloy.

Strowd's procedure was first tried on some aqueous extracts of cured tobacco leaf and found that with some samples it was not always possible to get satisfactory duplicates. It was thought possible that the prescribed conditions do not permit the hydrogen to completely reduce the nitrate during the preliminary heating and subsequent distillation. The modification described below was afterwards found to usually give higher and more concordant results than Strowd's method. The procedure used in this work and subsequently referred to as the modified method is as follows:

THE MODIFIED STROWD METHOD

Dilute two equal filtered portions of a cold-water extract of the sample to 250–350 cc. in 1-liter Kjeldahl flasks. Add a small piece of paraffin, a few small glass beads or pieces of pumice stone and 2.5–3 gm. of sodium hydroxid in solution (1:2) to each. To one solution add 2–3 gm. of Devarda alloy powder and use the other as a control. Place a rubber stopper with a Bunsen valve in the neck of each flask and let stand 24 hours, after which attach to a condenser carrying an improved form of distilling bulb (McHargue's form (*4*) was used in this work). Heat with a low flame just under boiling for 2 hours and then distill over at least 150 cc. The distillate is collected in a measured portion of 0.1 N sulphuric acid, the end of the outlet tube having been so placed as to dip beneath the acid from the beginning. Care should be taken that the control and determination be carried on in the same manner; that is, distilled at the same rate and same volume of distillate collected. The excess of acid in the receiving flask is then titrated with standard alkali, using an aqueous solution of sodium alizarin sulphonate as indicator. The difference between the two titrations corresponds to nitric plus nitrous nitrogen.

In this work, any small amounts of nitrite which may have been present are disregarded. It is doubtful if any quantity was present that would have materially influenced the nitrate results and besides this would not affect the comparisons between the two procedures. If its separation is desired, this can be made by a preliminary treatment with aspartic acid according to Strowd's directions and then following the above procedure. If relatively large amounts of nitrate

are to be determined, it is preferable to use the larger amounts of water and reagents specified and collect more distillate.

The principal change from Strowd's method is that the reduction is allowed to proceed for several hours before the solution is heated and the time of preliminary heating is increased. Some arrangement like the Bunsen valve permits the distilling apparatus to be utilized in the meantime for other purposes. The other modifications which prescribe a larger amount of alloy, a longer period of preliminary heating and larger volume of distillate, provide better conditions when relatively large amounts of nitrate are to be determined. The writer is of the opinion that the preliminary reduction before heating is the most important change made in Strowd's procedure, however, as excellent results have been obtained on some samples of moderate nitrate content by otherwise conforming to his directions.

Experiments have been made with the modified procedure to test the following points: (1) A possible loss of ammonia in the preliminary reduction; (2) its efficiency compared with the regular Strowd method for the reduction of relatively large amounts of pure nitrate; (3) the recovery of added nitrate in the presence of tobacco; (4) a comparison of the two procedures for nitrate in tobacco; (5) a comparison of the modified Strowd and the Ulsch-Street methods for nitrate in fertilizers and (6) a comparison of the nitrogen obtained in the control determinations by the modified Strowd and Ulsch-Street methods with that found by the official magnesium oxid method for ammoniacal nitrogen in fertilizers.

Blank determinations were made and deducted in reporting the results in the tables on all of the above work.

LOSS OF AMMONIA IN THE PRELIMINARY REDUCTION

For this work either Baker's analyzed ammonium sulphate or Eimer and Amend's tested ammonium chlorid were used. They were assumed to be pure, were practically moisture-free but no analyses were made to verify their composition inasmuch as they were to be used for a comparative study of the two methods for ammonia recovery. As the results obtained on both salts were equally close to the theoretical, no distinction is made in their discussion. For the determination, separate weighings of 0.4 gm. of the former salt or 0.3 gm. of the latter were used. These amounts are theoretically equivalent to 0.0848 gm. and 0.0785 gm. of nitrogen, respectively, representing more nitrate than would ordinarily be present in a determination. Control determinations were made by the modified method in which either the ammonium salt or Devarda alloy were omitted. When the ammonia salt was omitted the results were negligible, with or without the alloy. The recovery of ammonia from ammonium salts in the absence of the alloy is shown in Table I, C and E.

Determinations were also made by the modified method, connecting the flasks with the condenser instead of the Bunsen valve during the 24-hour digestion at room temperature. These are indicated in Table I, D and E. At the writer's request, duplicate determinations were made in the same manner by H. D. Spears, of this station, and his results were within the range given and are included in the averages under these headings in Table I.

The results by the modified method indicate a slightly better recovery of the ammonia by the use of the Bunsen valve compared with those where the flask was connected at once with the condenser as shown in B and D. Neither are as good, however, as those found by the Strowd method. In the determinations where the alloy was not used as shown in C and E, the results were just the reverse, in fact the average of those in E or with the condenser are better than those found by the Strowd method.

A titration of the acid through which the hydrogen gas from the digestion of the ammonium salt with sodium hydroxid and Devarda alloy had passed for 24 hours, before heat was applied, where the flask was connected with the condenser, showed a very small quantity of ammonia by a Nessler test. The amount obtained required less than 0.05 cc. of 0.1 N acid in the titration.

TABLE I.—*Percentage recovery of nitrogen from ammonium salts*

Method	No. of determinations	Maximum	Minimum	Average
A—Strowd method	4	98.4	97.8	98.1
B—Modified Strowd method, Bunsen valve	4	97.1	96.2	96.6
C—Same as B, but without Devarda alloy	3	97.9	97.1	97.4
D—Same as B, but connected with condenser [a]	12	97.6	92.9	95.7
E—Same as D, but without Devarda alloy	8	99.5	97.5	98.7

[a] Block tin condensers were used for all distillations. A battery of same was kindly furnished for this work by the Feed Control Laboratory of this station.

It would appear from Table I that for some unaccountable reason, the Devarda alloy had a detrimental effect on the ammonia recovery, as lower results were obtained in its presence.

THE EFFICIENCY OF THE TWO PROCEDURES FOR THE REDUCTION OF RELATIVELY LARGE AMOUNTS OF PURE NITRATE

Pure potassium nitrate and sodium nitrate were used and separate weighings of 0.25 gm. or 0.5 gm. were made, generally the latter amount. No material differences were found in the recovery of the nitrogen from either quantity and all are included in Table II. A comparison was again made by the modified method, connecting the flask with the condenser instead of the Bunsen valve during the 24-hour digestion at room temperature. The results are given in B and C of Table II.

TABLE II.—*Percentage recovery of nitrogen of pure nitrate*

Method	No. of determinations	Maximum	Minimum	Average
A–Strowd method	5	99.2	98.7	[a] 98.9
B–Modified Strowd method, Bunsen valve	8	99.4	97.4	98.4
C–Same as B, but connected with condenser	6	99.0	92.8	[b] 95.4

[a] One determination equivalent to 89.2 per cent recovery is not included.
[b] One determination equivalent to 90.9 per cent recovery is not included.

It will be observed that good results were obtained by both procedures on relatively large amounts of pure nitrate. The determinations by the modified method where the flasks were connected at once with the condenser are not as good as those with the Bunsen valve. This is contrary to what would be expected if there had been a loss of ammonia in the latter case.

THE RECOVERY OF ADDED NITRATE IN THE PRESENCE OF TOBACCO

The plan of this experiment was to digest the cured, pulverized tobacco in distilled water for 30 minutes by shaking every minute during the first and last five-minute periods. It was then filtered and two aliquots, each representing 0.7 gm. of tobacco were withdrawn. To one was added either 0.25 gm. or 0.5 gm. of sodium or potassium nitrate. Both determinations were made simultaneously and the difference in the nitric nitrogen obtained represents that recovered from the added nitrate. No material differences were found in the different quantities and kinds of nitrate used. Some determinations, also, were made by connecting the flasks at once with the condenser and these are so designated in the table. The tobacco samples were selected for their low nitrate content. Three contained 0.04 per cent and No. 80078 only 0.05 per cent nitric nitrogen. The results obtained are given in Table III.

TABLE III—*Recovery of added nitrate in the presence of tobacco*

Number	Strowd method	Modified Strowd method	
		With Bunsen valve	With condenser
	Per cent	*Per cent*	*Per cent*
80078	97.1	97.4	91.3
	95.2	98.3	88.1
		95.1	
		97.3	
		97.5	
		96.5	
Average	96.2	97.0	89.7
80236	98.5	94.2	89.2
		97.7	
Average		96.0	
80247	99.3	98.3	88.3
		97.2	
Average		97.8	
80274	98.4	97.5	97.5
		95.5	
Average		96.5	

A COMPARISON OF THE TWO PROCEDURES FOR NITRATE IN TOBACCO

Two aliquots prepared in the same manner as previously described, each representing 0.7 gm. of the cured sample, were withdrawn from the same filtered aqueous extract of the powdered tobacco. One aliquot was used for the determination and the other as a control, the latter having no Devarda alloy added. Some determinations, also, were made by the Strowd method in which the preliminary heating was for two hours instead of the prescribed one-hour period. The samples represent different grades of leaf, both of Burley and dark tobacco grown in Kentucky. The results obtained are given in Table IV.

TABLE IV.—*Nitrate nitrogen in moisture-free tobacco by the two procedures*

Number	Strowd method	Modified Strowd method
	Per cent	*Per cent*
61339	0.17	0.18
	.19	----
	.11	----
	.10	----
Average	.14	----
61340	*.11	.15
61341	*.11	.15
61342	*.02	.07
61343	*.17	.29
61344	*.04	.06
61345	*.05	.16
61347	*.22	.24
61348	*.03	.06
61349	*.16	.19
61350	*.01	.08
61351	*.03	.06
61352	*.11	.22
61375	.09	.26
	*.22	.31
		.31
		.26
Average	.16	.29
61376	.59	.85
	.77	.80
	*.78	.89
		.84
Average	.71	.85
61377	*.15	.20
61378	.24	.41
	*.24	----
Average	.24	----
61379	*.12	.20
61380	.33	.44
	*.31	.34
		.44
		.37
Average	.32	.40
80119	.95	.99
	.88	.99
Average	.92	.99
80125	1.54	1.36
	1.42	1.38
Average	1.47	1.37

* Preliminary heating was for 2 hours instead of 1 hour, as recommended.

TABLE IV.—*Nitrate nitrogen in moisture-free tobacco by the two procedures*—Con.

Number	Strowd method	Modified Strowd method
	Per cent	*Per cent*
80132	1. 23	1. 10
	1. 11	1 14
Average	1. 17	1. 12
80250	. 54	. 64
	. 61	. 63
Average	. 58	. 64
80273	. 49	. 54
	. 49	. 55
Average	. 49	. 55
80275	. 65	. 66
	. 66	. 70
Average	. 66	. 68
80276	. 87	. 89
	. 86	. 85
Average	. 87	. 87
80298	. 74	. 81
	. 76	. 83
Average	. 75	. 82
80332	1. 04	1. 06
	1. 06	1. 04
Average	1. 05	1. 05

Of the 28 samples used the modified method gave higher averages on 24, the same on 2, and lower on 2 than the other procedure.

COMPARISON OF THE MODIFIED STROWD AND ULSCH-STREET METHODS FOR NITRATE IN FERTILIZERS

The samples were obtained from the fertilizer department and were selected chiefly on account of their containing ammonium salts but no nitrate and part of their nitrogen in organic combination. All were complete fertilizers except Nos. 63902, 64026, and 64773, and these were, respectively, commercial nitrate of soda, commercial nitrate of lime, and pure bone meal. The total nitrogen content of the complete fertilizers varied from 0.91 per cent to 5.89 per cent and their organic nitrogen content from 0.47 per cent to 3.3 per cent. The object of selecting such samples was to find if the nitrogen of added nitrate could be recovered in their presence; if so, then this would afford a method for determining nitrate in the presence of ammonium salts and organic nitrogenous compounds. Moreover, it was thought that possibly the control determinations might also afford an accurate estimation of the ammoniacal nitrogen present.

This will be discussed later. Potassium nitrate was used and 0.25 gm. in solution was added in each determination, including the control.

When the filtered water extract of the sample was used the nitrate was added to this solution. Determinations were made on the whole sample and on the filtered aqueous extract of the same. In the latter aliquots were withdrawn from the same solution, one of which was used for the control. These are designated in the table as "Filtered." Those by the Ulsch-Street method were made in a similar manner and are likewise designated in the table. In all cases 0.7 gm. of fertilizer or aliquots representing this amount were used. The initial volume for the distillation by the modified Strowd method was 350 cc. and 3 gm. of Devarda alloy, together with the Bunsen valve, was used. The results are given in Table V.

TABLE V.—*Comparison of the modified Strowd and Ulsch-Street methods for nitrate in fertilizers*

Number	Modified Strowd method for recovery of added nitric N		Ulsch-Street method for recovery of added nitric N	
	Filtered	Not filtered	Filtered	Not filtered
	Per cent	*Per cent*	*Per cent*	*Per cent*
63595	98.6	95.2	----	93.9
	97.1	99.7	----	----
Average	97.9	97.5	----	----
63601	96.0	97.7	[a] 95.1	[a] 94.2
	98.8	----	----	74.0
Average	97.4	----	----	84.1
63605	98.3	95.2	----	92.5
	97.7	96.5	----	----
Average	98.0	95.9	----	----
63607	99.7	95.7	----	86.7
	97.4	98.6	----	----
	98.8	----	----	----
Average	98.6	97.2	----	----
63619	98.6	96.2	----	75.7
	98.6	99.7	----	----
Average	98.6	98.0	----	----
63621	98.0	99.1	----	77.2
		97.1	----	----
Average	----	98.1	----	----
63899	98.0	101.7	[a] 102.9	[a] 95.7
	98.3	----	----	84.5
Average	98.2	----	----	90.1

[a] Volume of solution was 350 cc. and collected 150 cc. of distillate. In the official method the prescribed volume is less than 150 cc. and only 100 cc. of distillate could be obtained. The solution of acid and iron was boiled for 10 minutes instead of 5 minutes as recommended.

TABLE V.—*Comparison of the modified Strowd and Ulsch-Street methods for nitrate in fertilizers*—Continued

Number	Modified Strowd method for recovery of added nitric N		Ulsch-Street method for recovery of added nitric N	
	Filtered	Not filtered	Filtered	Not filtered
	Per cent	*Per cent*	*Per cent*	*Per cent*
63902	[b] 15. 87	[c] 16. 24	----	[c] 16. 10
	[b] 15. 97	[c] 16. 10	----	[c] 15. 99
	[d] 15. 92	----	----	----
Average	----	16. 17	----	16. 05
64026	[b] 12. 80	[c] 12. 28	----	[c] 11. 92
	[b] 12. 42	[c] 12. 23	----	[c] 12. 19
	[d] 12. 28	----	----	----
Average	----	12. 26	----	12. 06
64773	100. 0	98. 8	[a] 90. 2	[a] 95. 1
	99. 1	97. 7	----	77. 5
Average	99. 6	98. 3	----	86. 3
64776	96. 8	97. 7	----	86. 1
	98. 9	99. 1	----	----
Average	97. 9	98. 4	----	----
64777	98. 3	98. 8	----	78. 0
	99. 7	94. 2	----	79. 2
Average	99. 0	96. 5	----	78. 6
64813	99. 1	97. 7	----	85. 3
	96. 5	97. 7	----	----
	98. 6	98. 8	----	----
Average	98. 1	98. 1	----	----
64828	98. 6	97. 1	----	82. 4
	95. 4	98. 3	----	----
Average	97. 0	97. 7	----	----

[b] Total nitrogen in original sample by Kjeldahl-Gunning-Arnold method was modified to include nitrate and $CuSO_4$ used but no Hg.
[c] Nitric nitrogen found in original sample by method indicated.
[d] Nitric nitrogen found in original sample by the fertilizer department according to the ferrous sulphate-zinc-soda method.

There was a slightly better recovery of the nitrate by the Strowd method but the results by both procedures are good except in those determinations where the flasks were connected at once with the condenser, in which case the results were lower. Here, as in the experiments where the nitrate alone was used, the results were contrary to what would be expected if there had been a loss of ammonia through the Bunsen valve.

The results show that a better recovery of the nitrate was made by the modified Strowd than by the Ulsch-Street method. A slight modification of the latter method to provide a better reduction and distillation proved of decided advantage. This is shown in those determinations where the reduction was for 10

minutes and a larger volume of distillate was collected. There were not very large differences found by the modified Strowd method in the percentages of nitrate recovered from the filtered and unfiltered solutions of tobacco. The advantage is slightly in favor of the filtered solution. Good results were obtained in either case, and this would indicate that it is not necessary to filter in order to make a satisfactory determination.

The modified Strowd and Ulsch-Street methods were found to be very satisfactory for commercial nitrates, but the latter method gave much lower results on the other samples except when the modification of it referred to above was employed, in which event more satisfactory results were obtained.

COMPARISON OF THE MODIFIED STROWD, ULSCH-STREET, AND OFFICIAL MAGNESIUM OXID METHODS FOR AMMONIACAL NITROGEN IN FERTILIZERS.

In this work the results obtained in the control determinations by the modified Strowd method on the fertilizer samples were compared with those found for ammoniacal nitrogen by the official magnesium oxid method. A more limited comparison was also made in the same manner by the Ulsch-Street method. (See Table VI.)

TABLE VI.—*Comparison of the modified Strowd, Ulsch-Street and official magnesium oxid methods for ammoniacal nitrogen in fertilizers*

Number	Official MgO method	Modified Strowd method		Ulsch-Street method	
		Filtered	Not filtered	Filtered	Not filtered
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*
63595	1. 12	1. 06	1. 25	----	1. 33
	[b] 1. 14	1. 11	1. 26	----	----
		1. 11	1. 26	----	----
		1. 02	----	----	----
Average	1. 13	1. 08	1. 26	----	----
63601	0. 28	. 29	. 50	[c] 0. 32	[c] . 44
	[b] 0. 21	. 31	. 53	----	. 45
		. 23	. 43	----	. 48
Average	0. 25	. 28	. 49	----	. 46
63605	0. 42	. 36	. 68	----	. 75
	[b] 0. 37	. 35	. 72	----	----
		. 35	. 64	----	----
		. 30	----	----	----
Average	0. 40	. 34	. 68	----	----
63607	1. 17	1. 21	1. 35	----	1. 37
	[b] 1. 24	1. 20	1. 33	----	----
		1. 25	1. 29	----	----
		1. 17	----	----	----
Average	1. 21	1. 21	1. 32	----	----

[a] Control computed as ammoniacal nitrogen.
[b] Results obtained by this method in the fertilizer department.
[c] Volume of solution was 350 cc. and collected 150 cc. of distillate. In the official method the prescribed volume is less than 150 cc. and only 100 cc. of distillate could be obtained. The solution of acid and iron was boiled for 10 minutes instead of 5 minutes as recommended.

TABLE VI.—*Comparison of the modified Strowd, Ulsch-Street and official magnesium oxid methods for ammoniacal nitrogen in fertilizers*—Continued

Number	Official MgO method	Modified Strowd method		Ulsch-Street method	
		Filtered	Not filtered	Filtered	Not filtered
		Per cent	*Per cent*	*Per cent*	*Per cent*
63619	0. 44	0. 41	0. 65	----	0. 64
	[b] 0. 44	. 37	. 62	----	----
		. 41	. 60	----	----
		. 40	----	----	----
Average	0. 44	. 40	. 62	----	----
63621	0. 78	. 81	. 89	----	. 87
	[b] 0. 76	. 85	. 89	----	----
		. 81	. 84	----	----
		. 81	----	----	----
Average	0. 77	. 82	. 87	----	----
63899	4. 54	4. 64	4. 60	[c] 3. 96	[c] 4. 54
	[b] 4. 48	4. 59	4. 72	----	4. 56
		4. 60	4. 72	----	----
Average	4. 51	4. 61	4. 68	----	4. 55
64773	0. 18	. 23	. 38	[c] . 21	[c] . 30
		. 26	. 40	----	. 33
		. 25	. 41	----	. 27
		. 13	. 27	----	----
Average	----	. 22	. 37	----	. 30
64776	0. 45	. 47	. 63	----	. 51
	[b] 0. 45	. 46	. 61	----	. 53
		. 44	. 55	----	----
Average	0. 45	. 46	. 60	----	. 52
64777	0. 79	. 85	1. 00	----	. 94
	[b] 0. 77	. 83	. 97	----	. 85
		. 83	1. 01	----	----
		. 82	. 92	----	----
Average	0. 78	. 83	. 98	----	. 90
64813	0. 96	. 95	1. 04	----	. 88
	[b] 0. 93	. 93	1. 04	----	1. 04
		. 92	1. 07	----	----
		. 91	1. 03	----	----
		. 91	----	----	----
Average	0. 95	. 92	1. 05	----	. 96
64828	0. 15	. 16	. 35	----	. 29
		. 17	. 29	----	----
		. 15	. 28	----	----
		. 13	----	----	----
Average	----	. 15	. 31	----	----

[b] Results obtained by this method in the fertilizer department.

[c] Volume of solution was 350 cc. and collected 150 cc. of distillate. In the official method the prescribed volume is less than 150 cc. and only 100 cc. of distillate could be obtained. The solution of acid and iron was boiled for 10 minutes instead of 5 minutes as recommended.

The results obtained for ammoniacal nitrogen by the modified Strowd method show that it is necessary to filter the solution, after which the aliquot for the control can be used for the ammonia determination. In fact the duplicates agree as well as the determinations made by different analysts by the official method on the same sample.

The distillation with magnesium oxid for ammoniacal nitrogen often gives high results on materials containing organic nitrogen. As the averages obtained by the modified Strowd method for this determination agree with the official procedure consequently they together with the nitrate results indicate that it is possible to make a satisfactory estimation of both ammonia and nitrate on the same solution provided a filtered aqueous extract of the sample is employed. If only the nitrate is desired, filtration will probably not be necessary although it may be desirable under different conditions.

The writer does not recommend the modified Strowd procedure for all nitrate determinations until further work establishes its reliability for the purpose. At the same time, it is believed that it will be found useful for samples of a different nature from those employed in this investigation.

SUMMARY

1. A comparison has been made of a Devarda alloy method published by Strowd for the determination of nitrates in plants and a modification of same in which it was found advantageous to let the reduction continue for twenty-four hours at room temperature, previous to heating the solution.

2. Both procedures show a fairly complete recovery of considerable amounts of pure nitrate in solution and of nitrate added to tobacco of a low nitrate content.

3. More concordant and generally higher results were obtained, however, by the modified Strowd method when nitrates were determined in different kinds of tobacco of variable nitrate content.

4. The modified method was also found to be applicable for the estimation of nitrate in commercial fertilizers. No determinations were made by the regular Strowd procedure on these samples.

5. The control determination which is necessary as a check in the modified procedure was found to be as accurate for the determination of ammoniacal nitrogen in fertilizers as the official magnesium oxid method.

6. If both ammonia and nitrate are to be determined from aliquots of the same aqueous extract of a sample, it is necessary to filter from the residue. Where nitrate alone was determined in the samples used in this work, previous filtration proved to be of small advantage.

7. The modified Strowd method is not recommended for general application for nitrate determinations until its reliability is established, but it is believed that its use can be extended to others of a different nature from those employed in this investigation.

LITERATURE CITED

(1) Association of Official Agricultural Chemists.
1920. Official and tentative methods of analysis. As compiled by the Committee on Revision of Methods. Revised to Nov. 1, 1919. 417 p., illus. Washington, D. C.

(2) Caron, H.
1912. Dosage des nitrates dans l'urine. Ann Chim. Analyt. 9: 9–13.

(3) CHAMOT, E. M., PRATT, D. S., and REDFIELD, H. W.
1911. A MODIFIED PHENOLSULFONIC ACID METHOD. Jour. Amer. Chem. Soc. 33: 381–384.

(4) MCHARGUE, J. S.
1919. A NEW FORM OF DISTILLING BULB. Jour. Indus. and Engin. Chem. 11: 670–671.

(5) POZZI-ESCOT, E.
1909. RÉDUCTION DE L'AZOTE NITRIQUE À L'ÉTAT D'AZOTE AMMONIACAL ET NOUVEAU PROCÉDÉ DE DOSAGE DES NITRATES. Ann. Chim. Analyt. 14: 445–446.

(6) STROWD, W. H.
1920. THE DETERMINATION OF NITRITES AND NITRATES IN PLANT TISSUE. Soil Sci. 10: 333–342.

(7) TREADWELL, F. P.
1919–21. ANALYTICAL CHEMISTRY. Tr. by W. T. Hall. Ed. 5. v. 2. New York.

PUCCINIA GRAMINIS POAE ERIKSS. AND HENN. IN THE UNITED STATES[1,2]

By E. C. STAKMAN, *Plant Pathologist, Division of Plant Pathology and Botany, Department of Agriculture, University of Minnesota, and Pathologist, Office of Cereal Investigations, Bureau of Plant Industry, United States Department of Agriculture*, and M. N. LEVINE, *Pathologist, Office of Cereal Investigations, Bureau of Plant Industry, United States Department of Agriculture.*

Eriksson and Henning (*4*),[3] in 1896, first described *Puccinia graminis poae* on *Poa chaixi* Vill. Eriksson (*2*) later showed that it could infect *Poa caesia* Smith, *P. compressa* L. and *P. nemoralis* L., and that aecia were formed on *Berberis vulgaris* L. The following grasses were inoculated by Eriksson but did not become infected: *Briza media* L., *Festuca elatior* L., *F. ovina* L., *F. pilosa* Haller, *Hordeum vulgare* L., *Koeleria cristata* var. *gracilis* A. Gray and *Phleum boehmeri* Wibel. Jaczewski (*5*) recorded the occurrence of this form on *Poa compressa* L. in Russia, and stated that it also could infect *P. pratensis* L. and *P. serotina* Ehrh., but not *Agropyron repens* (L.) Beauv., *Avena sativa* L., *Bromus inermis* Leyss., *B. secalinus* L., *Dactylis glomerata* L., *Hordeum vulgare* L., *Secale cereale* L., and *Triticum vulgare* Vill. Both Eriksson and Jaczewski called *P. graminis poae* "scharf fixiert" and Eriksson designated it as "Isophage," because it was confined to a single genus, *Poa*.

The writers have not been able to find records of the occurrence of *P. graminis poae* in the United States. Neither Carleton (*1*) nor Stakman and Piemeisel (*14*) mentioned it; and the writers did not find it during the course of extensive field observations previous to 1923. Several specimens of what appeared to be *P. graminis* on *Poa* spp. have been sent to the writers, but the amount of rust always was small and no infection was obtained as a result of artificial inoculations until inoculations were made with material obtained from Michigan in the fall of 1922.

In the fall of 1922 Donald G. Fletcher and Walter F. Reddy observed very heavily infected *Poa compressa* near barberry bushes in the vicinity of Pontiac, Mich., and sent material to the writers for identification. There were few urediniospores on the plants, the rust being mostly in the telial stage.

In order definitely to establish the identity of the rust inoculation experiments were made. On account of the paucity of uredinial material it was necessary to start with teliospores. The results of these experiments are given in Table I. In the summer of 1923 plants heavily infected with the uredinial stage were collected and additional inoculations made, the results of which are given in Table II.

[1] Received for publication March 12, 1924

[2] Cooperative investigations between the Office of Cereal Investigations, Bureau of Plant Industry, United States Department of Agriculture, and the Department of Agriculture of the University of Minnesota. Published with the approval of the Director, as Paper No. 426 of the Journal Series of the Minnesota Agricultural Experiment Station.

[3] Reference is made by number (italic) to "Literature cited," pp. 547–548

TABLE I.—*Results of inoculating Berberis vulgaris and various grasses and cereals at University Farm, St. Paul, Minn., with Puccinia graminis poae, from Poa compressa collected near Pontiac, Mich., in 1922* [a]

Original inoculum	Species and varieties inoculated—			
	March 21, 1923	April 26, 1923	May 18, 1923	June 5, 1923
Teliospores from *Poa compressa*.	*Berberis vulgaris*... 4—	*Poa annua*... 0 *P. compressa* 4— *P. nemoralis* 0 *P. pratensis*. 4 *P. triflora*... 0	*Poa annua*... 0 *P. compressa*... 4— *P. nemoralis*... 4 *P. pratensis*... 4 *P. triflora*... 0 *Avena sativa* (Victory, Minn. 514) [b]... 0 *Hordeum vulgare* (Manchuria, Minn. 105)... 0 *Secale cereale* (Rosen, Minn. 82)... 0 *Triticum compactum* (Little Club, C.I. 4066)... 0	*Poa compressa*... 4 *Agrostis alba*... 0 *Dactylis glomerata*... 0 *Secale cereale* (Rosen, Minn., 82)... 0

[a] The sequence of inoculations in Tables I and II is indicated by dashes, proceeding from left to right. All of the hosts in the same vertical column were inoculated with spores from the host immediately preceding the dashes. The degree of infection is indicated after each plant inoculated: 0=no infection; 1=extremely light infection; 3=moderate infection; 4=very heavy infection. The date above each vertical column is that on which the inoculations were made.

[b] Accession numbers of Minnesota Agricultural Experiment Station, and Office of Cereal Investigations, Bureau of Plant Industry, U. S. Department of Agriculture, respectively.

TABLE II.—*Results of inoculating Poa spp., Phleum pratense, and cereals with urediniospores of Puccinia graminis poae from Poa compressa collected near Pontiac, Mich., in 1923*

Original inoculum	Species and varieties inoculated—			
	August 23, 1923	September 6, 1923	September 28, 1923	October 18, 1923
Urediniospores from *Poa compressa*.	*Poa annua*... 3 *P compressa* 3— *P. fertilis*... 3 *P. nemoralis*... 3 *P. triflora*... 3 *Avena sativa* (Victory, Minn. 514)... 0 *Phleum pratense*... 0 *Triticum compactum* (Little Club, C.I. 4066) [a]... 0	*Poa compressa* 4— *P. pratensis*. 4—	*Poa compressa*... 4— *Avena sativa* (Victory, Minn. 514) [a]. 0 *Hordeum vulgare* (Manchuria, Minn. 105)... 1 *Phleum pratense*... 0 *Secale cereale* (Rosen, Minn. 82)... 0 *Triticum compactum* (Little Club, C. I. 4066)... 0 *Poa compressa*... 4 *Phleum pratense*... 0	*Poa compressa*. 3 *P. pratensis*... 4

[a] Accession numbers of Minnesota Agricultural Experiment Station, and Office of Cereal Investigations, Bureau of Plant Industry, U. S. Department of Agriculture, respectively.

It will be seen from Tables I and II that *Poa annua* L., *P. compressa* L., *P. fertilis* Host, *P. nemoralis* L., *P. pratensis* L., and *P. triflora* Gilib. are susceptible to the rust. Very heavy infection always resulted from inoculating *P. compressa*. While the infection on the other species of Poa was normal, it was not so heavy as that on *P. compressa*. *Agrostis alba* L., *Dactylis glomerata* L., and *Phleum pratense* L. did not become infected, so the rust could not have been either *P. graminis agrostis* or *P. graminis phleipratensis*. In addition, the fact that *Berberis vulgaris* was very susceptible also excluded the possibility of the rust being *P. graminis phleipratensis*. As wheat, oats, and rye apparently are immune, and barley nearly so, the rust could not be the *tritici*, the *avenae* or the *secalis* variety. It therefore must be *P. graminis poae*.

The host range of *P. graminis poae* appears to be narrow. According to Eriksson (*2* and *3*) and Jaczewski (*5*), this rust variety is restricted to the genus *Poa*. If this is true it is the only variety of *P. graminis* now known to occur in the United States which infects species of a single genus only. However, it probably can infect, at least weakly, members of other genera, because minute uredinia developed on barley (Table II). Extensive inoculations are now under way in order to ascertain the infection capabilities of the rust.

Morphologically, also, the variety *poae* is quite distinct, the spores being characteristically small. The urediniospores, teliospores and aeciospores are considerably shorter than those of any other variety, but they do not differ so much in width. Tables III and IV present the measurements of the three kinds of spores of *P. graminis poae* and a comparison of these with those of the other varieties of *P. graminis*.

TABLE III.—*Variations and constants for length and width of the various spores of Puccinia graminis poae grown on congenial host plants and under favorable environmental conditions*

Kinds of spores	Spore diameters	Spore classes							n	Mean	Standard deviation	Coefficient of variability
Teliospores from *Poa compressa*...	Length	20μ	30μ	40μ	50μ	60μ			100	36.90±0.62	9.13±0.44	24.74±1.25
		8	35	40	14	3						
	Width	11μ	13μ	15μ	17μ	19μ	21μ		100	15.52±0.14	2.07±0.10	13.34±0.65
		4	19	35	33	7	2					
Aeciospores from *Berberis vulgaris*.	Length	12μ	13μ	14μ	15μ	16μ	17μ	18μ	100	15.07±0.09	1.35±0.06	8.96±0.43
		2	11	20	31	21	11	4				
	Width	11μ	12μ	13μ	14μ	15μ			100	13.23±0.07	0.97±0.05	7.33±0.35
		5	14	43	29	9						
Urediniospores from *Poa compressa*.....	Length	15μ	17μ	19μ	21μ	23μ			100	18.64±0.10	1.51±0.07	8.10±0.39
		1	35	46	17	1						
	Width	13μ	14μ	15μ	16μ	17μ	18μ		100	15.78±0.07	1.06±0.05	6.72±0.32
		3	12	14	48	21	2					

TABLE IV.—*Summary of differences between the means of the dimensions of the various spores of Puccinia graminis poae and those of other varieties of Puccinia graminis*

Diameters.	Biologic forms compared. [a]		Teliospores		Aeciospores		Urediniospores	
			Difference in means (in microns)	Difference divided by P. E.	Difference in means (in microns)	Difference divided by P. E.	Difference in means (in microns)	Difference divided by P. E.
Length	*Puccinia graminis poae* and	*P. graminis tritici*	14.90±0.79	18.86	4.65±0.21	22.14	13.76±0.21	65.52
		P. graminis secalis	10.45±0.77	13.57	2.03±0.21	9.67	8.50±0.17	50.00
		P. graminis avenae	9.25±0.75	12.33	3.55±0.14	25.36	9.86±0.18	54.78
		P. graminis phleipratensis	4.40±0.70	6.29			5.31±0.16	33.19
		P. graminis agrostis	3.40±0.74	4.59	1.39±0.20	6.95	3.73±0.16	23.31
Width	*Puccinia graminis poae* and	*P. graminis tritici*	1.15±0.18	6.39	2.43±0.12	20.25	4.01±0.09	44.56
		P. graminis secalis	0.75±0.18	4.17	0.20±0.13	1.54	1.41±0.09	15.67
		P. graminis avenae	0.32±0.18	1.78	1.47±0.12	12.25	4.16±0.10	41.60
		P. graminis phleipratensis	0.11±0.17	0.65			1.10±0.09	12.22
		P. graminis agrostis	0.88±0.18	4.89	0.25±0.11	2.27	0.10±0.09	1.11

[a] Data on forms other than *P. graminis poae* from Levine (*6*).

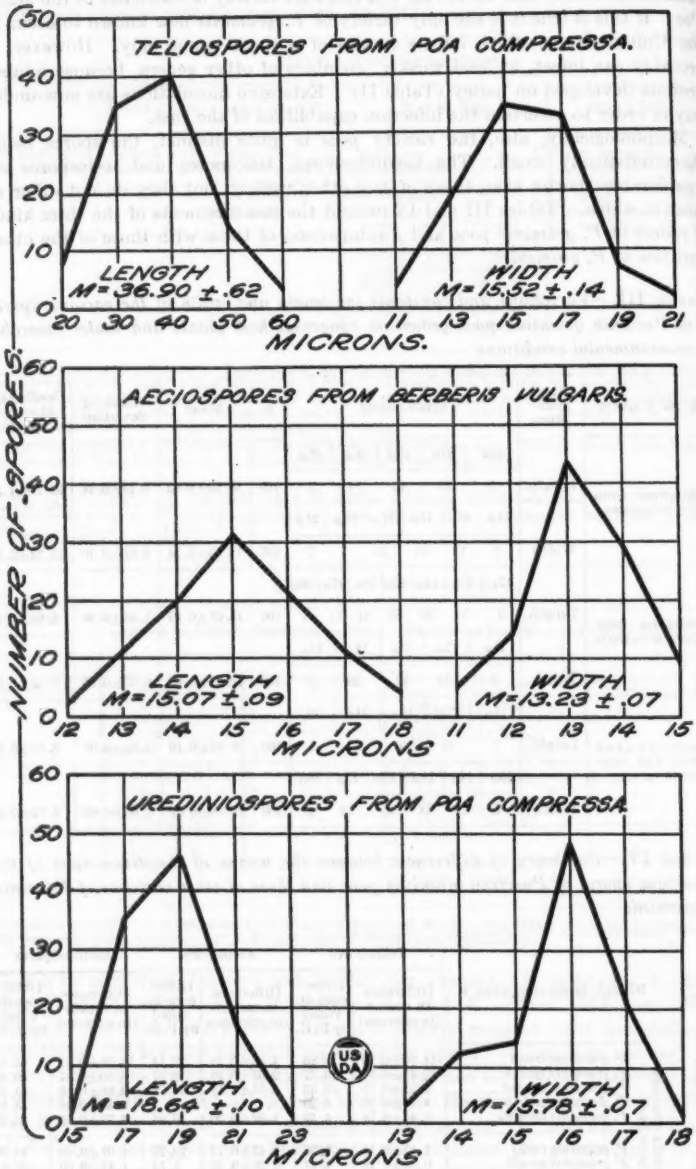


FIG. 1.—Variations in the size of spores of *Puccinia graminis poae*

The mean length for teliospores of *P. graminis poae* is 3.40±0.74μ less than that for those of *P. graminis agrostis*, which formerly were considered the shortest of all. This difference is 4.59 times the probable error and therefore is significant statistically. According to the computations of Pearl and Miner (*8*), the odds against the occurrence of such a difference on account of random sampling are about 520 to 1. The mean length for aeciospores of *P. graminis poae* is only 1.39±0.20μ less than that for those of the variety *agrostis*. But this difference is even more significant than the apparently greater difference between the means of the length of the teliospores of these varieties, because it is almost 7 times its probable error, and the odds against its chance occurrence are about 40,000 to 1. Of still greater significance is the difference between the means of the length of the urediniospores of these two varieties. It is more than 23 times its probable error, the actual difference being 3.73±0.16μ.

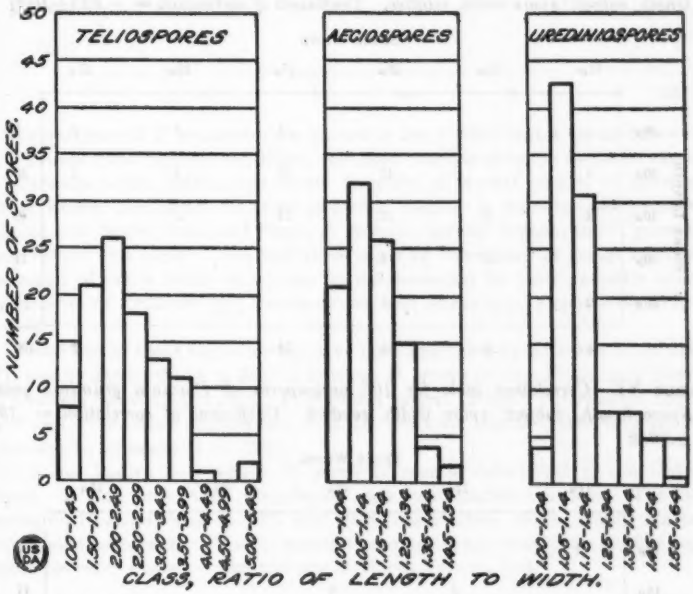


FIG. 2.—Ratios of length to width of the various spores of *Puccinia graminis poae* arranged in classes

The mean width of the teliospores of the *poae* variety is approximately the same as that of the varieties *avenae* and the *phleipratensis*, that of the aeciospores is similar to the mean width of the varieties *secalis* and *agrostis*, and that of the urediniospores is nearly the same as that of *P. graminis agrostis*.

The variations in length and width of the various spores of *Puccinia graminis poae* are shown graphically in fig. 1.

The ratio of length to width, as shown in fig. 2, was determined by the method suggested by Rosenbaum (*10*, p. 250). The correlations of length and width of the three different kinds of spores are presented in Tables V, VI, and VII.

The teliospores (Pl. 1, B) of the variety *poae* appear to be extremely variable in shape. Some are very short and broad (27.14×20.01μ) while others are greatly elongated and narrow (63.48×11.73μ). The predominating ratio of length to width of the teliospores is about 2 to 1. There is a definite negative correlation (−.613±.0421) between the long and short diameters of these spores (Table V).

The aeciospores (Pl. 1, D) are predominantly spherical to subspherical in shape, the modal length-to-width ratio being approximately 1.1 to 1. The thickening of the upper wall, in vertical section of the sorus, described by Rosen and Kirby (*9*) as characteristic of the aeciospores of *P. graminis*, was quite evident. There is some indication of a positive correlation (.194±.0649) between the length and width of these spores (Table VI), but it probably is too small to be considered statistically significant.

There is clearly no correlation (.038±.0673) between the two diameters of the urediniospores (Table VII). These spores are for the most part subglobose (Pl. 1, F), resembling the urediniospores of *P. graminis avenae* on *Bromus tectorum* L. (*11*, p. 62), and the predominating length-to-width ratio is about 1.1 to 1.

TABLE V.—*Correlation table for 100 teliospores of Puccinia graminis poae. Spore length, subject; spore width, relative. Coefficient of correlation = −.613±.0421*

Spore Length	Spore Width 11μ	13μ	15μ	17μ	19μ	21μ	
20μ				5	2	1	8
30μ	1		13	17	3	1	35
40μ	1	9	17	11	2		40
50μ		10	4				14
60μ	2		1				3
	4	19	35	33	7	2	100

TABLE VI.—*Correlation table for 100 aeciospores of Puccinia graminis poae. Spore length, subject; spore width, relative. Coefficient of correlation = .194 ±.0649*

Spore Length	Spore Width 11μ	12μ	13μ	14μ	15μ	
12μ	1	1				2
13μ		2	9			11
14μ		3	9	8		20
15μ	2	4	11	11	3	31
16μ	1	3	10	5	2	21
17μ	1	1	3	4	2	11
18μ			1	1	2	4
	5	14	43	29	9	100

TABLE VII.—*Correlation table for 100 urediniospores of Puccinia graminis poae. Spore length, subject; spore width, relative. Coefficient of correlation = .038 ±.0673*

Spore Length	Spore Width 13μ	14μ	15μ	16μ	17μ	18μ	
15μ		1					1
17μ	2	2	9	18	4		35
19μ	1	4	1	25	13	2	46
21μ		4	4	5	4		17
23μ		1					1
	3	12	14	48	21	2	100

The varieties of *P. graminis* now known in the United States are as follows:[4] *P. graminis tritici* Erikss. and Henn. (actually consists of many forms or races), *P. graminis secalis* Erikss. and Henn. (consists of several forms), *P. graminis avenae* Erikss. and Henn. (consists of several forms), *P. graminis phleipratensis* (Erikss. and Henn.) Stak. and Piem., *P. graminis agrostis* Erikss., and *P. graminis poae* Erikss. and Henn. The first three must be considered as group varieties, consisting of many forms which can be differentiated by their parasitic action on varieties of *Triticum* spp., *Secale cereale*, and *Avena* spp., respectively (*12*, *7*, and *13*).

In addition to these the following have been described by Eriksson (*3*, p. 370–371) and by Jaczewski (*5*, p. 353) in Europe: *P. graminis airae* Erikss. and Henn., *P. graminis calamagrostis* Jacz. (*P. graminis epigeii* Erikss.), *P. graminis aperae* Jacz. and *P. graminis arrhenatheri* Jacz. [*P. graminis avenae* Erikss. and Henn., according to Eriksson (*3*, p. 370)].

It is not known how widely *P. graminis poae* is distributed in the United States. It has been found abundantly only near barberry bushes. The epidemics on *Poa compressa* in 1922 and 1923 near Pontiac, Mich., clearly started from rusted barberries. Heavily rusted *P. compressa* also was found in 1923 near rusted barberry bushes in the vicinity of North Vernon, Ind.[5]

LITERATURE CITED

(1) CARLETON, M. A.
1899. CEREAL RUSTS OF THE UNITED STATES: A PHYSIOLOGICAL INVESTIGATION. U. S. Dept. Agr. Div. Veg. Phys. and Path. Bul. 16, 74 p., illus.

(2) ERIKSSON, J.
1902. UEBER DIE SPEZIALISIERUNG DES GETREIDESCHWARZROSTES IN SCHWEDEN UND IN ANDEREN LÄNDERN. Centbl. Bakt. (II) 9: 590–607, 654–658.

(3) ———— 1918. FORTGESETZTE STUDIEN ÜBER DIE SPEZIALISIERUNG DES GETREIDE-SCHWARZROSTES (PUCCINIA GRAMINIS) IN SCHWEDEN UND IN ANDEREN LÄNDERN. Centbl. Bakt. (II) 48: 349–417.

(4) ———— and HENNING, E.
1896. DIE GETREIDEROSTE . . . 464 p., illus. Stockholm.

[4] For list of host plants see Stakman and Piemeisel (*14*).

[5] Specimens and information furnished by F. J. Eliason and W. F. Graham, field assistants, Office of Cereal Investigations, Bureau of Plant Industry, U. S. Department of Agriculture.

(5) JACZEWSKI, A. von.
1910. STUDIEN ÜBER DAS VERHALTEN DES SCHWARZROSTES DES GETREIDES IN RUSSLAND. Ztschr. Pflanzenkrank. 20: 321–359, illus.

(6) LEVINE, M. N.
1923. A STATISTICAL STUDY OF THE COMPARATIVE MORPHOLOGY OF BIOLOGIC FORMS OF PUCCINIA GRAMINIS. Jour. Agr. Research 24: 539–568, illus.

(7) ——— and STAKMAN, E. C.
1923. BIOLOGIC SPECIALIZATION OF PUCCINIA GRAMINIS SECALIS. (Abstract) Phytopathology 13: 35.

(8) PEARL, R., and MINER, J. R.
1914. A TABLE FOR ESTIMATING THE PROBABLE SIGNIFICANCE OF STATISTICAL CONSTANTS. Maine Agr. Exp. Sta. Bul. 226: 85–88.

(9) ROSEN, H. R., and KIRBY, R. S.
1919. A COMPARATIVE MORPHOLOGICAL STUDY OF AECIA OF FOUR DIFFERENT RUSTS FOUND UPON BARBERRIES IN NORTH AMERICA. Phytopathology 9: 569–573, illus.

(10) ROSENBAUM, J.
1917. STUDIES OF THE GENUS PHYTOPHTHORA. Jour. Agr. Research 8: 233–276, illus.

(11) STAKMAN, E. C., and LEVINE, M. N.
1919. EFFECT OF CERTAIN ECOLOGICAL FACTORS ON THE MORPHOLOGY OF THE UREDINIOSPORES OF PUCCINIA GRAMINIS. Jour. Agr. Research 16: 43–77.

(12) ——— ———
1922. THE DETERMINATION OF BIOLOGIC FORMS OF PUCCINIA GRAMINIS ON TRITICUM SPP. Minn. Agr. Exp. Sta. Tech. Bul. 8, 10 p., illus.

(13) ——— ——— and BAILEY, D. L.
1923. BIOLOGIC FORMS OF PUCCINIA GRAMINIS ON VARIETIES OF AVENA SPP. Jour. Agr. Research 24: 1013–1018, illus.

(14) ——— and PIEMEISEL, F. J.
1917. BIOLOGIC FORMS OF PUCCINIA GRAMINIS ON CEREALS AND GRASSES. Jour. Agr. Research 10: 429–496, illus.

III, No. 4

FREIDEN
s.

OF BIO-
rch 24:

(Ab-

STATIS-
3.

DIFFER-
Phy-

arch 8:

OGY OF
gr. Re-

INIS ON
llus.

AVENA

RASSES.

PLATE 1

Puccinia graminis poae on congenial host plants, and photomicrographs of the different spore forms (all photomicrographs x 500).

A.—Heavily rusted *Poa compressa* from the field; the rust is in the telial stage.

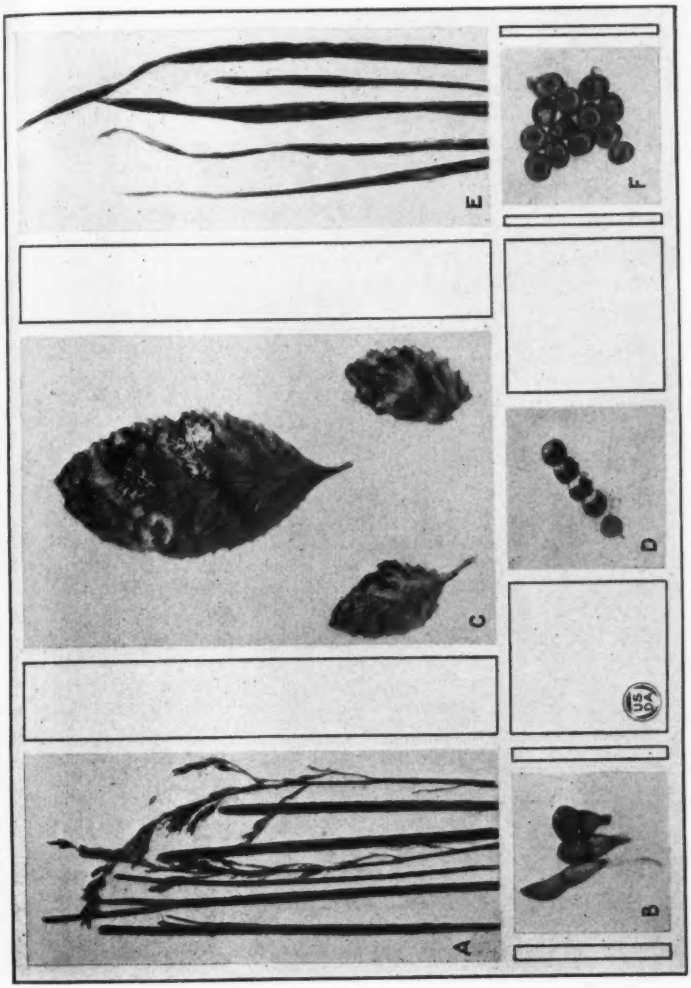
B.—Teliospores from A.

C.—Aecia on *Berberis vulgaris* resulting from artificial inoculation in the greenhouse; note the long aecial cups.

D.—Aeciospores from C.

E.—*Poa compressa* heavily infected with the uredinial stage as a result of artificial inoculation with urediniospores in the greenhouse.

F.—Urediniospores from E.


E
F
C
D
A
B

RELATION OF SOIL TEMPERATURE AND SOIL MOISTURE TO INFECTION BY PLASMODIOPHORA BRASSICAE[1]

By JOHN MONTEITH, Jr.

Assistant Pathologist, Office of Cotton, Truck, and Forage Crop Disease Investigations, Bureau of Plant Industry, United States Department of Agriculture

INTRODUCTION

The peculiar nature of the clubroot disease of crucifers has focused upon it the attention of both mycologists and plant pathologists ever since Woronin first proved (*21*)[2] that it is caused by the parasitic slime mold. As a result there is an extensive literature dealing with both the scientific and the practical aspects of this malady. The life history of the organism is now very well understood and the recent studies by Kunkel (*12*) seem to give a satisfactory explanation of its relation to the host tissue. These more fundamental scientific studies seem to have proceeded more satisfactorily than those dealing with control measures. Following the early lead of Halsted (*7*, *8*) American phytopathologists have generally relied on the use of lime as an adequate control measure, and Ravn (*14*, *15*) has been an earnest advocate of liming for this disease in Europe. The results, while justifying the use of lime, have not proven uniformly reliable in either America (*9*) or Europe. Moreover plants that show early infection will sometimes recover (*6*) and produce a satisfactory crop.

The evidence seems to indicate that while soil reaction is important in governing infection with this parasite there must be other factors exercising an important and, perhaps at times, a controlling influence upon either its entrance into the host tissue or upon the subsequent development of the disease. It seems evident, therefore, that a fuller and more exact understanding of the factors influencing infection and the progress of the disease may contribute both to our understanding of the physiological relation of parasite and host and to the furthering of reliable control measures.

In this disease we are dealing with an organism which persists for some time in the soil and the attacks of which are practically confined to the underground host organs. The recent studies at Wisconsin (*11*, *19*) upon the influence of environment upon other root diseases suggest that soil temperature and soil moisture may be expected to play an important part in determining the occurrence or severity of such diseases. The present paper deals with the results of efforts to determine the influence of these two factors.

EARLIER OBSERVATIONS

Differences in the prevalence and severity of clubroot have frequently been attributed to variations in "climatic conditions," but few attempts have been made to analyze these conditions as to their effect upon the disease. Clubroot is reported as causing most damage in northern regions of the United States and Europe, from which it might be inferred that temperature plays an important part in its distribution. It has long been recognized that affected plants are more likely to wilt and die in periods of hot, dry weather during July and August, than at other times. This has been interpreted as the result of increased trans-

[1] Received for publication February 1, 1924.

[2] Reference is made by number (italic) to literature cited, p. 559–561.

piration at a time when the roots could not supply sufficient moisture because of the distortions due to the presence of the organism in their tissues. In interpreting this evidence it should be kept in mind that the parasite probably made its entry early in the host's development when the soil was relatively cool and moist, and that the disease had progressed to an advanced stage before the dry, hot period of midsummer. Halstead (*7*, *8*) after noting the greater destructiveness of clubroot on turnips during a hot season comments as follows:

> The extreme heat of midsummer, although detrimental to the normal development of turnips, seemed to have an opposite effect upon the parasite preying upon their roots. When harvested, the turnips were found to be distorted to a much greater degree than any grown upon the same land since the experiment began.

These observations, although directing attention to temperature, were based upon field plots where the comparisons were between different seasons in which other factors may have varied greatly.

Chupp (*5*) studied the earlier stages of the disease under controlled conditions and from his observations concluded that temperature might play an important part in spore germination and infection. In discussing his results on spore germination he states that—

> Temperature conditions also influence germination of the spores. It was practically impossible to obtain infection in the greenhouse during the coldest winter months when the temperature was from 10° to 18° C. The spores also fail to germinate at ordinary room temperatures (from 16° to 21° C.) The optimum temperature for germination proved to be from 27° to 30° C. This, however, is not the case when spores are placed in test tubes on agar with young cabbage seedlings, for under such conditions infection takes place at a temperature of from 16° to 21° C. The presence of the host seems in some manner to exert an influence which to a certain extent takes the place of that offered by a greater amount of heat.

Chupp's results indicate that studies upon the germination of spores apart from the plant will not necessarily apply to conditions as they exist in the soil. The introduction of cabbage seedlings indicates that his first results were influenced more by some unfavorable condition of his germinating medium than by temperature. This seems to be the only case in which temperature has been definitely designated as a limiting factor in the development of clubroot.

The influence of soil moisture upon clubroot development has been given more attention by previous workers than has temperature. However, in these cases no one has clearly differentiated the possible influence of moisture from the other variable factors such as soil reaction, aeration, or humus content. Chupp (*5*), Reed (*18*), and Ravn (*14*) cite cases where the disease was more severe in the low areas of the field, and this accords with observations in southern Wisconsin. Drainage of such areas was recommended by Ravn (*13*) who cited cases where the disease had been checked by proper drainage alone. Anderson (*1*) reports a similar case of control by drainage, although in some instances he found the land became more subject to the disease after being drained. Halsted (*7*, *8*) observed that watering increased the virulence of the disease during a dry season. Whitehead (*20*), Reed (*18*), Cunningham (*6*) and Ravn (*15*) noted that the disease was more severe after periods of wet weather. Humphrey (*10*) working in Massachusetts found the disease worse on the heavy and moist soils, while Reed (*18*) in Virginia notes that—

> In soils which contain a large amount of organic matter (humus) and are warm and moist, the organisms find conditions very suitable for their growth.

Ravn, who, with his associates, conducted field experiments in Denmark for many years, gave more critical attention to the possible influence of soil moisture on the disease than any previous worker had. However, his conclusions were all

based upon observations with no direct experimental work. He frequently found a severe attack of the disease associated with heavy rainfall. In 1903 (*16*) he refers to a plot in which turnips were planted just previous to a heavy rain. The plants came up well, but had a sickly appearance from the start and were uniformly infected even where lime had been applied.. In this same plot two years later he observed that the disease first appeared after a heavy rain of several days' duration and spread rapidly thereafter. In summarizing his observations on the influence of different seasonal conditions upon the occurrence of the disease (*13*) he was able to find no indication of a uniform severity or absence of the disease in any given area over a period of several years. He cites a number of cases where the disease was more severe on low ground, although he often attributes this to other causes in addition to excessive soil moisture. He reports (*14*) one plot which was planted on low ground where considerable water remained from fall to spring, although it was artificially drained. In this plot he observed that infection was most severe in the lower portions, which he attributed to excessive soil moisture and recent cultivation of turnips on the area. In another publication (*13*) he states that other things being equal, the disease is worst on the low parts of the field with wet, cold, and "sour" soil. He quotes 23 cases observed in 1905 and 1906, all of which show that the more moist the soil, the more malignant the infection. In a survey of plant diseases for 1916 and 1917 he again reports (*17, p. 312*) a case on low ground where infection was severe. Although this land had never been in cultivation before, only 6 of the 300 plants produced heads, all the others being destroyed by clubroot, probably in association with maggots. He also found that land which was poorly drained favored the development of the disease as did the low areas.

He refers to one plot (*14*) which had poor drainage where infection was unusually malignant at the close of August and continued to destroy the plants until harvest. The almost total destruction of the stand, notwithstanding the application of lime in the experiment, he considered as due to several cooperating circumstances, chief of which were probably deficiency of lime and defective drainage for he points out that on adjoining property having the same type of soil but which was well drained and marled there was little or no infection. Two other plots of this series at times showed severe infection in parts where drainage was not adequate, and he thought that this factor was partly responsible for the presence of the disease in this extreme form. He wrote (*13*) of a case where the disease had been increasing in severity for a number of years on an area which needed drainage. In 1901 the soil was drained, and the destruction due to clubroot was considerably reduced and had not increased since then in spite of the fact that cultivation of cabbage had been continued. He concluded that the moisture conditions of the soil were so important that all precautions against the disease are valueless when drainage is not perfect. He thus modified his recommendation of lime as a control for clubroot. After the long series of observations with Christensen and Harder (*4*) he recommends for control of this disease, a correction of acidity by liming. But where the soil does not need lime and the fungus is still present, he suspects defective drainage or some unfavorable physical condition of the soil and advises that these conditions be improved. He was convinced (*16*) that on well-drained arable land where there is sufficient calcium carbonate the introduction of the clubroot fungus will not cause any serious infection. As an explanation of the effect of weather conditions on the occurrence of clubroot, he seemed inclined to believe that an abundant precipitation at the time when the myxoamoebae swarm in the soil will favor infection in a high degree, but he offers no proof of this. Conversely, he states that a warm, dry period in later stages of the disease may make it worse, since he frequently noticed infected turnip and rape plants wilting in

dry weather whereas in moist summers the disease often caused little damage, even when a large part of the plants were infected.

While Ravn's observations seem clearly to justify his conclusions that, under the soil conditions prevailing in Danish fields, soil drainage, along with soil reaction, plays an important part, yet he gives no exact or experimental data upon which to base comparisons. He did not carry out any experiments to determine definitely the influence of water content apart from the other factors connected with poor drainage. While he follows the common habit of speaking of low or poorly drained soils as "cold," we have no data on the possible influence of soil temperature as a factor in relation to clubroot infection or development. It seemed, therefore, worth while to attempt to segregate and define more clearly the possible influence of each soil temperature and soil moisture on this disease.

METHODS

Unless otherwise stated under the individual experiments, the method of procedure was in general as outlined below. In most of this work two distinct types of infested soil were used, both being in good physical condition and of high fertility. These will be designated throughout as A and B. Soil A was a clay loam obtained from a small home garden located at Chatham, N. J., where the disease had become serious and widespread. Soil B was a sandy loam, rich in organic matter, taken from a badly diseased portion of a commercial cabbage field near Kenosha, Wis. The water-holding capacity was determined for each soil by means of the usual 12-inch brass cylinder method. For comparison with these determinations the moisture equivalents were determined by means of the method described by Briggs and McLane (*2*), that is, by using a centrifuge with a radius of six inches and a speed of 2,400 revolutions per minute for 40 minutes. The wilting coefficient for each soil was calculated by means of the indirect method based on the moisture equivalent as described by Briggs and Shantz (*3*). The following is a summary of these determinations:

Water-holding capacity, moisture equivalent, and wilting equivalent of the clubroot infested soils

Soil	Water-holding capacity	Moisture equivalent	Wilting coefficient
	Per cent	*Per cent*	*Per cent*
A	45	24	13
B	62	34	19

To insure vigorous growth of plants the fertility of the soil was in each case increased by adding powdered potassium nitrate and dicalcium phosphate at the rate of 1 gm. of each chemical for every 3 kgm. of soil. Each lot was sifted through a 3-mm. sieve and the water content determined. Cans of equal weight were selected, and an equal weight of soil was put into each one so that when packed and planted the surface was about 1 inch below the top of the can. In order to make provision for water and aeration an inverted 3-inch flower pot connected with the surface by a glass tube was placed near the center of each can. When the soil was mixed, the water content was a little above that desired as the lowest moisture content to be maintained in the series. When a given series was repeated, the same soil was sifted, mixed, and replaced in the cans.

In all this work seedling cabbage plants were used. These were grown in sterilized soil and transplanted to 3-inch pots where they were allowed to grow for from three to eight weeks in a greenhouse maintained at a temperature

ranging between 15° and 20° C. The variety Copenhagen Market was used in the first series and Wisconsin Hollander in all subsequent series. Both varieties appeared equally susceptible to clubroot. Plants were selected as nearly equal in size and general vigor as possible for all cans of each series. The soil was carefully removed from the roots and all long roots were broken off to a length of 2 inches before transplanting into the diseased soil. Four plants were set in each can and the soil pressed firmly about the roots. The experiments were all conducted in the same greenhouse, and each series was arranged so that the light, air, temperature, and humidity would be as nearly equal as possible for all plants.

The cans used for the soil temperature series were 6 inches in diameter and 10 inches deep. In all the temperature series the soil in half the number of cans was adjusted to a high moisture content, and the remainder was kept at a low moisture content. The water in the wet soil was added after transplanting to avoid danger of "puddling" the soil around the roots at the time of transplanting. In the experiment in which the effect of soil moisture alone was studied, cans 5 inches by 5 inches were used. The procedure of preparing the soil, filling, and planting was essentially the same as that used for the temperature studies except that a circular glass plate 2 inches in diameter was used in place of the inverted pot. Water was added to these cans twice daily to avoid the necessity of adding very large amounts at one time.

The plants were grown in the cans of infested soil for periods of three to eight weeks, depending on the age when planted and on the rate of growth. In general, they were grown until the plants in some of the cans were severely injured by the disease and had become badly wilted. In removing the plants care was taken to preserve intact as many of the roots as possible. However, since the amount of naturally infested soil available was limited, it was necessary to sacrifice part of the smaller roots to avoid losing too much soil. Since in all cases infection was most severe on or near the stem or main roots, the loss of the smaller roots did not materially diminish the value of the results.

SOIL TEMPERATURE IN RELATION TO CLUBROOT INFECTION

For the soil temperature experiments the cans were placed in "Wisconsin soil temperature tanks" which have been described fully elsewhere (*11*). By this method the aerial parts of all plants in the same experimental series were exposed to like atmospheric conditions while the soil temperatures for the various plants were varied according to the needs of the experiment. The soil moisture was also varied with the experimental culture cans.

SERIES I: In the first series four tanks were maintained at 10°, 18°, 24°, and 30° C., respectively. Plants of commercial Copenhagen Market variety were used. Since the soil contained the organism of the yellows disease, *Fusarium conglutinans*, which is stimulated by high temperatures, most of the plants at the higher temperatures were badly injured by the latter disease. After three weeks they were removed and discarded since the presence of the two diseases rendered confusing results. However, since severe clubbing occurred on all plants grown at 18° C., while none were affected at 10°, the next series was maintained at temperatures of 9°, 12°, 15°, and 18° C. in order to determine the lowest temperature at which the disease would occur.

SERIES II: In this series two cans each of A and B soils were used for each temperature. The soil in one can was kept at a water content of approximately 35 per cent, and the soil in the other at 64 per cent of its water-holding capacity. After a period of one month the plants were removed and the roots examined. It was found that at the three higher temperatures uniform clubbing developed

on all plants in soil with the higher moisture content, but that only scattered infection occurred in soils with the lower moisture content. No case of clubbing was found in the soil kept at 9° C., even at the higher moisture.

SERIES III: Since the plants grown in soil maintained at 12° C. showed severe clubbing, only temperatures below 16° were included in later series with these soils. By doing this the soil was kept below the temperature range for infection by the Fusarium. Therefore, the third series was run in tanks maintained at 6°, 9°, 12°, and 15° C. As soil moisture appeared to be exerting a marked influence on the disease, the range in water content was increased. Soil A was maintained at 75 per cent and 30 per cent while soil B was maintained at 90 per cent and 30 per cent of the water-holding capacity. Since the plants were transplanted when considerably smaller than those used in previous experiments, they were allowed to grow for a longer period, approximately eight weeks. Upon examination of the roots it was found that in both soils with high moisture content all the plants were badly clubbed at 12° and 15° C. No cases of infection were found in either soil at 6° C. At 9° the plants grown in soil A showed no evidence of infection but each of the plants in the corresponding can of soil B in the same tank showed slight clubbing (Plate 3). Plate 2 shows the effect of these conditions on plants grown in clean soil. In no case of low soil moisture (either A or B) was there any evidence of clubbing at any temperature. Plate 1 shows one representative plant from each can of soil A at the higher moisture content.

SERIES IV: In order to determine the influence of higher temperatures, without confusion with yellows disease, four lots of soil were secured infested with *Plasmodiophora brassicae* but free from *Fusarium conglutinans*. One sample was obtained from the same garden as soil A and kept free from contamination with the Fusarium in the greenhouse. The other three were from Vermont, New York, and Minnesota, respectively,[3] from fields where clubroot was known to occur, but where yellows had not been observed. These soils were used for planting in tanks at 20°, 25°, 30°, and 35° C. The plants used in this series were larger than those in the previous temperature series and the period of growth in the infested soil was shorter.

Soil A was used at a water content of 30 and 75 per cent as in the lower temperature series. No clubbing occurred at any temperature in the soil maintained at 30 per cent of the water-holding capacity. In the soil maintained at 75 per cent of the water-holding capacity severe clubbing occurred at 20° C. but there appeared to be a reduction in the amount and severity of the disease as the temperature rose. Since there was a tendency for the soil to become dry more quickly at the higher temperatures due to the fact that the cans used for this series were smaller, and the plants were larger, it is possible that these conditions contributed to the results. The soils from Vermont, Minnesota, and New York were used in small cans (5 inches in diameter and 5 inches in depth) and kept at a moisture content of about 75 per cent of the water-holding capacity. The large plants, together with the limited amounts of water in these small volumes of soil and the fact that no insulation was used, made it so difficult to hold the moisture constant, especially at the higher temperature, that the results were not considered final. However, they were uniform in showing that infection was most severe at 20° C. and decreased as the temperature rose. Clubbing in all parts of the root system occurred at all these higher temperatures except 35°, in which case it occurred only on the main stem at the surface of the soil where its contact with the air may have resulted in a somewhat lower temperature.

[3] These were obtained through the courtesy of B. F. Lutman, Chas. Chupp, and E. C. Stakman, respectively.

SERIES V. In order that a comparison of the temperature influence over the whole range might be obtained in a single series, the lot of soil A which was free from *Fusarium conglutinans* was mixed with one-fourth its volume of quartz sand to make enough for 16 of the 6-inch cans. These cans were planted with plants more than two months old which had been somewhat checked in growth by being grown in small pots. The moisture was kept at 75 per cent of the water-holding capacity, and mineral wool insulation was used on the surface of the soil. Duplicate cans were placed in tanks kept at 6°, 9°, 12°, 15°, 20°, 25°, 30°, and 35° C. Wilting due to clubroot first occurred at 25°, and soon afterwards at 20° C. The growth of plants in these two temperatures was checked completely by the time the plants were removed. But plants at 15° and 30° seemingly were not checked in any way. Growth was very slight in the 35° tank. Failure to maintain the original low temperatures concluded the experiment before the disease had time to develop to the extremes which it might have attained in a more prolonged period. The plants were so large when transplanted that the relative growth of the tops did not indicate the actual effect of the disease and therefore were omitted from the photograph. The roots gave the best indication of the effect of the various temperatures upon development of the disease. Plate 4 shows two representative root systems taken from each temperature. The greatest development of clubs occurred at 20° and 25° C. There was also some clubbing at 15° and 30°, but none at the highest temperature 35° nor at the lower end of the series, 6°, 9°, and 12°. The results of this experiment are thus in accord with previous results.

The interpretation of the above results must take into account the effect of temperature on the host as well as on the organism. While temperature may limit infection by its direct effect upon the organism, it is obvious that with such a disease as clubroot its severity, as estimated by size of clubs, will depend to a great extent on the growth of the host immediately after infection. Tisdale (*19*) in his studies of the relation of soil temperature to the development of cabbage roots and tops has shown that the greatest root development occurs at about 20° C., with decided reduction above 25°; the present results are in accord with his findings. With regard to infection it appears from these results that clubroot infection will occur over nearly as wide a range as that in which growth of cabbage occurs. At the higher points the limits seem nearly to coincide. At the lower end of the range there seems to be a point (below 9° C.) at which infection does not occur and the host does grow very slowly. Between these two points (35° C. and 9° C.) there is a great difference in the severity of the disease. The largest clubs occurred at 20° C. which is close to the optimum temperature for root development. It may, therefore, be said that clubroot infection may occur at any temperature which is favorable for growth of cabbage and that the disease develops most rapidly and becomes most severe at about the optimum temperature for root development of this particular host plant.

In the individual series all plants were selected for uniformity of size and vigor; all were of the same age. However, there was a difference in age, size, and vigor between the different series. In general, it was observed that infection was less severe and development of clubs was slower in the plants that were older and less vigorous. Plants that had been temporarily checked before transplanting into the infested soil were slower in starting a vigorous growth in the new soil, and the disease was correspondingly delayed and less severe. This slow growth may explain some of the results of earlier attempts to produce the disease on old plants which led to the belief that infection occurred only on plants at the seedling stage. Kunkel (*12*) concluded that "old plants are almost as susceptible as young ones, provided they are growing." Although no plants

that could properly be called old were used in this work, it was noted that checking growth in general delayed development of the clubs. From the nature of the development of the organism within the host this dependence on host development seems probable.

SOIL MOISTURE IN RELATION TO CLUBROOT INFECTION

In a preliminary test, using ordinary flower pots placed on a greenhouse bench and watered with a sprinkling can, there was a wide variation in amount of clubbing in the different pots, indicating the influence of soil moisture since other factors were equal for all pots.

Series I. In the more carefully conducted temperature series, however, watering was done with greater care, using 6-inch cans maintained at high and low soil moistures, as described in Series II and IV above. All of the plants grown in soil with the low water content remained entirely free from disease, while all of those grown in the same soil with the higher water content became diseased within the favorable temperature range. Plate 1 shows a representative plant chosen from each can of the higher moisture series in soil A. Plate 3 represents the corresponding series in soil B. An examination of Plates 2 and 3 will show some difference in plant development due to difference in water content in the cans of soil where they were grown. Undoubtedly the plants grow more vigorously in soil with the higher moisture, provided no clubbing occurs to check growth. However, it is seen that in soil containing insufficient moisture for disease development the plants could obtain enough water for considerable growth. All plants were of equal size when planted. Those grown at 6°, especially those in the low-moisture cans, made little growth and may be used for comparison to indicate the relative amount of growth in cans with different known moisture content.

Series II. To test further the influence of soil moisture on clubroot a series was prepared with cans containing an amount of water equal to 30, 45, 60, 75, and 105 per cent of the water-holding capacity of the soil in the can. Plants in the soil containing only 30 per cent of the water-holding capacity were not protected for a long enough period to enable them to become established; as a result they wilted and died. Those in the soil containing water to the extent of 45 per cent of its water-holding capacity had little difficulty in becoming established and grew well. The plants in the two cans with higher water content wilted badly during the day for some time before they were taken out. When they were removed, it was found that clubs had developed on all the plants except those in the 45-per cent can. The roots of the plants in the 105-per cent cans were almost completely decayed. Those in the 90-per cent can had become so soft that they could not be taken out of the soil without breaking, but the others were still firm. Plate 5 shows one plant from each can and illustrates well the dwarfing of the plants at the higher moisture content and the relative clubbing in each.

From the results of these investigations it seems that the disease develops only occasionally, if at all, in soils with a water supply less than half of the water-holding capacity. This limit is, no doubt, somewhat higher or lower in soils rich or poor in organic matter. With amounts of water above this minimum there is apparently a direct ratio between severity of the disease and increase in water content. At the higher moisture content it is probable that the severity of the disease is not limited to development of the clubs but is also due to a tendency of the clubbed roots to succumb more promptly consequent to their invasion by secondary organism. The optimum water content for development of cabbage plants is in general about 75 per cent of the water-holding capacity of the soil. This influence of excessive moisture in stimulating the clubbing

is apparently the same throughout the range of temperatures at which the disease is capable of development. It is noteworthy that whereas in the case of temperature the influence upon disease development was correlated with that upon host growth, by contrast in the case of moisture this does not obtain. In soils that are saturated with water growth of cabbages is practically stopped. The stimulating influence can be explained partly by the fact that excessive soil moisture favors the activities of the parasite and partly by the fact that it increases the damage due to secondary decay of the clubs. The disease may develop on the roots without severely checking the growth of the plant when the soil moisture is limited, but when the soil moisture is excessive the diseased roots are soon destroyed by secondary decay organisms and the plant dies.

The host tissue in tops and roots of plants grown in soil with this low water content appears to develop normally, although perhaps not quite as vigorously as that in soil with more available moisture. Some plants grown in infested soil at the low moisture content, on which the disease did not appear at the conclusion of the experiment, were grown in nutrient solutions for a period of three weeks longer after having the soil carefully washed from their roots. On these plants no galls developed whereas similar plants on which galls had started while growing in the wetter soil continued to develop the clubs after being placed in the nutrient solution. Therefore, it seems likely that the disease was avoided by preventing penetration of the organism rather than by checking the growth of the gall. After attempting to germinate spores on the surface of agar, Chupp (*5*) reports that "unless the spores were immersed in water there was no development." It is probable that failure of the disease to develop on plants grown in infested soils with low moisture content is due to insufficient moisture for spore germination. It has been observed that extended desiccation will check the viability of spores and prolonged periods of drought may destroy the organism in the soil.

RELATION OF TEMPERATURE TO FIELD CONDITIONS

The indications are that under field conditions temperature is not a factor limiting the appearance of the disease, although it may exert an important influence on the severity of the disease if other factors are favorable.

Although the water content of the soil below which clubroot will not develop is insufficient for the most vigorous development of cabbage, it nevertheless provides moisture adequate for moderate growth. Under field conditions it is probable that there is frequently not sufficient soil moisture in the upper layer of soil to favor infection, and healthy plants at this time probably depend more on the deeper roots for their supply of water.

With these results in view it is interesting to note experiments conducted by Cunningham (*6*) in Vermont on the effect of "hilling up" cabbage plants as a method of controlling clubroot. He reports a method suggested by a Vermont grower, which consisted of hilling up the soil around the diseased plants to induce them to send out adventitious roots above the clubbed region. In explanation of the process, Cunningham notes that "The adventitious roots formed as a result of this practice on the stem above the diseased root are relatively disease free and function in the place of the affected roots which are practically useless." Further, he notes that "any procedure which induces club-free roots to form above the diseased roots greatly lessens the damage caused by the club." He conducted experiments during the course of two years in order to observe the effect of this procedure. "Decidedly beneficial results were secured in 1912, but in 1913 a decided injury was done." He, therefore, concludes that "hilling is most likely to prove helpful when the disease is most severe."

He also included observations on seasonal rainfall which aid in interpreting his results. He states that in 1913 the seasonal rainfall was only two-thirds of that of 1912, although both were below normal. The rainfall in 1912 was more evenly distributed and there was more cloudy weather. This was supposed to have stimulated the formation of adventitious roots. From this information we may infer that in 1912, the year in which clubroot was most severe, the soil was kept more moist by the even distribution of rainfall and by cloudy weather. Roots, therefore, probably became diseased in the flat soil which was moist enough to favor infection. The hilling process would provide drainage for the top layer of soil and probably reduced the moisture below the point where infection could occur. The adventitious roots developed in this hilled soil escaped infection because of the low moisture content of the soil immediately surrounding them. Enough moisture was present, however, so that the plants developed heads. The dry season of 1913 gave different results, probably because of excessive drying out of the soil beyond the point where roots could get enough moisture for growth. "The 1912 yield on some hilled plots was increased more than tenfold."

Similar development of adventitious roots was observed by the writer during the summer of 1920 in the Racine, Wis., district. Plants were badly clubbed in parts of some fields during the early growing period, and on hot days early in August these showed decided wilting. There was so little rainfall after July 1, that the soil became dry even in the lower areas. Adventitious roots, sent out from the stems above the clubbed areas, grew down into the soil beside the older, badly clubbed roots. These new roots did not become infected and in time were able to supply sufficient moisture to produce marketable heads; which were, of course, much below the average weight of heads produced on uninfected plants. It has been frequently observed in cabbage fields in various localities that when clubroot occurs regularly that it is usually most severe in the lower areas or depressions in a field. The continual favorable moisture conditions in such areas makes it possible for the organism to develop whenever wild or cultivated crucifers grow there and so the organism becomes abundant in such soil. On the other hand epidemics of the disease occur on higher and well drained soils which may be explained by the fact that the spores of this organism are known to be long lived and when the soil is once infested it only needs a season of abundant rainfall to make the disease severe even on this type of soils.

SUMMARY AND CONCLUSIONS

It has long been recognized that clubroot is influenced by various environmental conditions. In recent years the work on this disease has been largely with the influence of soil acidity and the effect of liming. This has been especially true with studies in America, where liming has become the principal means of control. However, it has been frequently observed that the disease is not checked by liming the soil. These results indicate that there are factors other than soil acidity which largely influenced the severity of the disease, so experiments were conducted to try to determine what these factors might be. The work here reported shows the effect of soil temperature and of soil moisture as related to the occurrence of clubroot.

The trials were made by planting cabbage seedlings in naturally infested soil in the greenhouse. The air conditions were alike for all plants but the soil conditions were varied experimentally. The earlier trials aimed to determine the possible influence of different soil temperatures upon disease development, the later ones to determine the possible influence of different soil moistures.

In the temperature series using the Wisconsin tank method with soil temperatures, ranging by gradation from 6° to 35° C. clubroot developed throughout the entire range except at the extremes, that is, from 9° to 30° C. Within this range, 9° to 30° C., the effect of temperature upon the clubbing seemed directly correlated with its influence on the growth of the host tissues. The most severe injury to the plant occurs at a temperature somewhat above that for largest club production, wilting occurring sooner and dwarfing of the plant being more noticeable at 25 than at 20° C. The conclusion reached therefore is that temperature is probably not a direct limiting factor in clubroot development under field conditions.

On the other hand soil moisture was found to be an important factor. The trials included a soil-moisture range graduated from 30 per cent of the water-holding capacity of the soil to full saturation. The disease developed on plants grown in soil maintained at a moisture content of 60 per cent of the water-holding capacity and all higher moistures but did not develop in soil kept at 45 per cent or less of capacity. Injury to the plants increased with an increase in soil moisture above 60 per cent. Plants, however, are able to develop well (although not at their best capacity) with a soil moisture of only 45 per cent of capacity.

It is probable that failure of the disease to develop on plants grown in infested soils with low moisture content is due to insufficient moisture for spore germination. This may be due to an absence of enough water to provide a film around the spore, a condition which appears (*5*) to be necessary for spore germination. Prolonged periods of drought may even destroy the organism in the soil.

An important factor under conditions of excessive moisture is that of secondary decay. The disease may develop on the roots without severely checking the growth of the plant when the soil moisture is limited, but when the soil moisture is excessive the diseased roots are soon destroyed by secondary decay organisms and the plant dies.

Under natural field conditions a long period during which the soil is so dry that no clubbing, or only small clubs, would develop, may give the plants an opportunity to produce a good crop regardless of short wet periods. Such periods of low soil moisture occur during many seasons in most of our cabbage-growing sections and this no doubt explains the difference in severity of the disease in the same field in different seasons.

On comparing previous literature on clubroot with the evidence here presented as to the influence of soil temperature and soil moisture it seems probable that a consideration of these factors, and especially of soil moisture, may help to explain the conflicting results secured concerning the use of lime and the relation of soil acidity to the clubroot disease.

LITERATURE CITED

(1) Anderson, A.
1855. Report on the disease of finger and toe in turnips. *In* Trans. Highland and Agr. Soc. Scotland, ser. 3, v. 6, p. 118–140.

(2) Briggs, L. J., and McLane, J. W.
1907. The moisture equivalents of soils. U. S. Dept. Agr. Bur. Soil Bul. 45, 23 p., 1 fig., 1 pl.

(3) ——— and Shantz, H. L.
1912. The wilting coefficient for different plants and its indirect determination. U. S. Dept. Agr. Bur. Plant Indus. Bul. 230, 83 p., 9 fig., 2 pl.

(4) CHRISTENSEN, H., HARDER, P., and RAVN, F. K.
1909. UNDERSØGELSER OVER FORHOLDET MELLEM JORDBUNDENS BESKAFFENHE OG KAALBROKSVAMPENS OPTRAEDEN I EGNEN MELLEM AARHUS OG SILKEBORG. *In* Tidsskr. Landbr. Planteavl., Bd. 16, p. 430–476.

(5) CHUPP, C. C.
1917. STUDIES ON CLUBROOT OF CRUCIFEROUS PLANTS. N. Y. Cornell Agr. Exp. Sta. Bul. 387, p. 417–452, illus. Bibliography p. 451–452.

(6) CUNNINGHAM, G. C.
1914. STUDIES OF CLUBROOT. II. DISEASE RESISTANCE OF CRUCIFERS; METHODS OF COMBATTING CLUBROOT. Vt. Agr. Exp. Sta. Bul. 185, p. 67–96, 9 pl. Bibliography, p. 95, 96.

(7) HALSTED, B. D.
1894. CLUBROOT OF CABBAGE AND ITS ALLIES. *In* N. J. Agr. Exp. Sta. 14th Ann. Rpt., 1893, p. 332–345, illus.

(8) ———
1895–1902. EXPERIMENTS WITH TURNIPS. *In* N. J. Agr. Exp. Sta. Ann. Rpt. 15th, 1894, p. 278–285; 16th, 1895, p. 250–267; 17th, 1896, p. 294–309; 18th, 1897, p. 265–274; 19th, 1898, p. 292–299; 20th, 1899, p. 354–367; 21st, 1900, p. 410–413; 22d, 1901, p. 388; illus.

(9) HASKELL, R. J., and WOOD, J.
1920. DISEASES OF FIELD AND VEGETABLE CROPS IN THE UNITED STATES IN 1919. U. S. Dept. Agr., Bur. Plant Indus., Plant Disease Bul. Supl. 10, p. 180–273. (Mimeographed.)

(10) HUMPHREY, J. E.
1892. THE CLUBROOT OF CABBAGES. *In* Mass. Agr. Exp. Sta. 9th Ann. Rpt., 1891, p. 230, 231.

(11) JONES, L. R.
1922. EXPERIMENTAL WORK ON THE RELATION OF SOIL TEMPERATURE TO DISEASE IN PLANTS. *In* Trans. Wis. Acad. Sci., Arts, and Letters, v. 20, p. 433–459, illus., pl. 33–37.

(12) KUNKEL, L. O.
1918. TISSUE INVASION BY PLASMODIOPHORA BRASSICAE. *In* Jour. Agr. Research, v. 14, p, 543–572, 2 fig., pl. 61–80. Literature cited, p. 571, 572.

(13) RAVN, F. K.
1908. KAALBROKSVAMPEN. *In* Tidsskr. Landbr. Planteavl., Bd. 15. p. 527–620, 4 pl.

(14) ———
1910. FORSØG MED ANVENDELSE AF KALK OG KUNSTGØDNING SOM MIDDEL MOD KAALBROKSVAMP. *In* Tidsskr. Landbr. Planteavl, Bd. 17, p. 163–177.

(15) ———
1911. FORSØG MED ANVENDELSE AF KALK SOM MIDDEL MOD KAALBROKSVAMP. *In* Tidsskr. Landbr. Planteavl, Bd. 18, p. 357–392.

(16) ———
1911. ET INFEKTIONSFORSØG MED KAALBROKSVAMP. *In* Biol. Arbejder Tilegnede Eug. Warming, 1911, p. 167–174.

(17) ———
1919. OVERSIGT OVER HAVEBRUGSPLANTERNES SYGDOMME I 1916 OG 1917. *In* Tidsskr. Landbr. Planteavl, Bd. 26, p. 298–334.

(18) REED, H. S.
1911. CABBAGE CLUB ROOT IN VIRGINIA. Va. Agr. Exp. Sta. Bul. 191, 12 p., 5 fig.
(19) TISDALE, W. B.
1923. INFLUENCE OF SOIL TEMPERATURE AND SOIL MOISTURE UPON THE FUSARIUM DISEASE IN CABBAGE SEEDLINGS. *In* Jour. Agr. Research, v. 24, p. 55–86, 10 fig., 2 pl. Literature cited, p. 85–86.
(20) WHITEHEAD, T.
1922. PLANT DISEASES. *In* Rpt. Exp. Univ. Coll. N. Wales, 1920–21, p. 40–50.
(21) WORONIN, M.
1878. PLASMODIOPHORA BRASSICAE, URHEBER DER KOHLPFLANZEN-HERNIE. *In* Jahrb. Wiss. Bot. [Pringsheim], Bd. 11, p. 548–574, pl. 29–34.

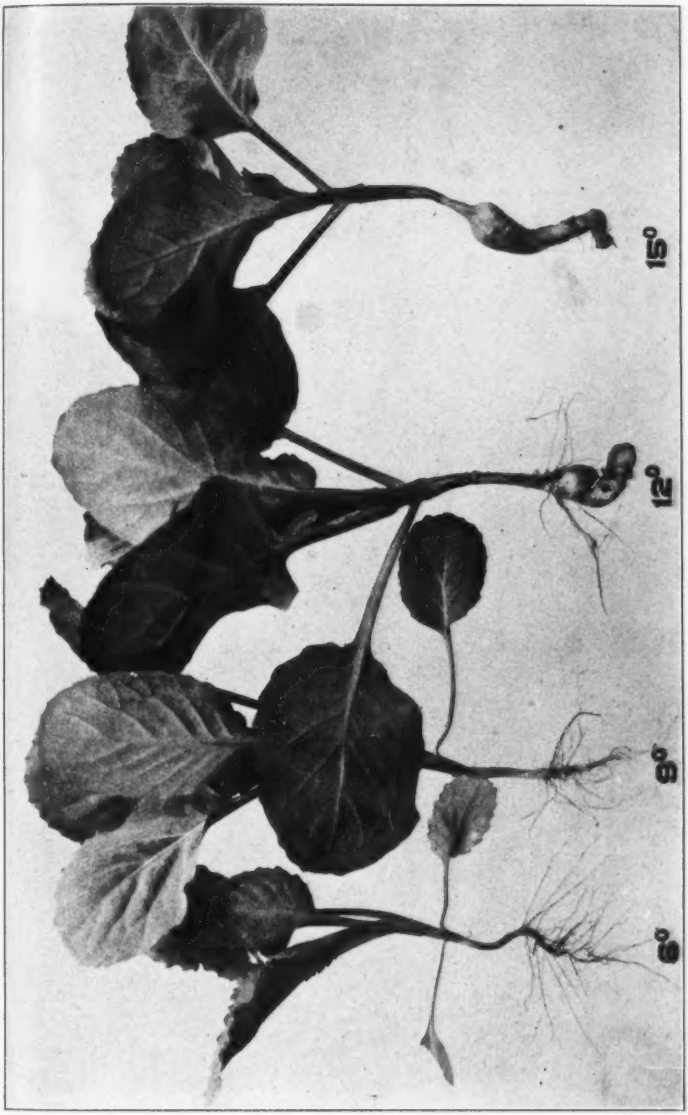
PLATE 1

INFLUENCE OF SOIL TEMPERATURE ON CLUBROOT OF CABBAGE

Note absence of disease at lower temperatures 6° and 9°, and the clubs at the higher temperatures, 12° and 15° C.

Plants grown eight weeks, at the soil temperatures indicated in a clay loam soil (soil A of the text, p. 552) uniformly infested with the clubroot organism. The air temperature for all alike was about 15° C. This soil was of a nature favorable to the development of clubroot and was kept uniformly at a moisture content found favorable for the development of the disease, that is 75 per cent of the water-holding capacity. The complete absence of clubroot in the two left-hand specimens must therefore be attributed solely to the inhibiting influence of soil temperature.

The relative size of these plants also deserves consideration. Note that normally at this range of soil temperatures with rising temperature there would occur a corresponding continued increase in the size of the seedlings (see Plate 2). Such normal increase in size is shown in the healthy seedling at 9° C., as compared with that at 6° C. The plant at 12° has evidently suffered some check as a result of the clubbing of its roots, and that at 15° has been much stunted because of the early and severe infection.


15°
12°
9°
6°

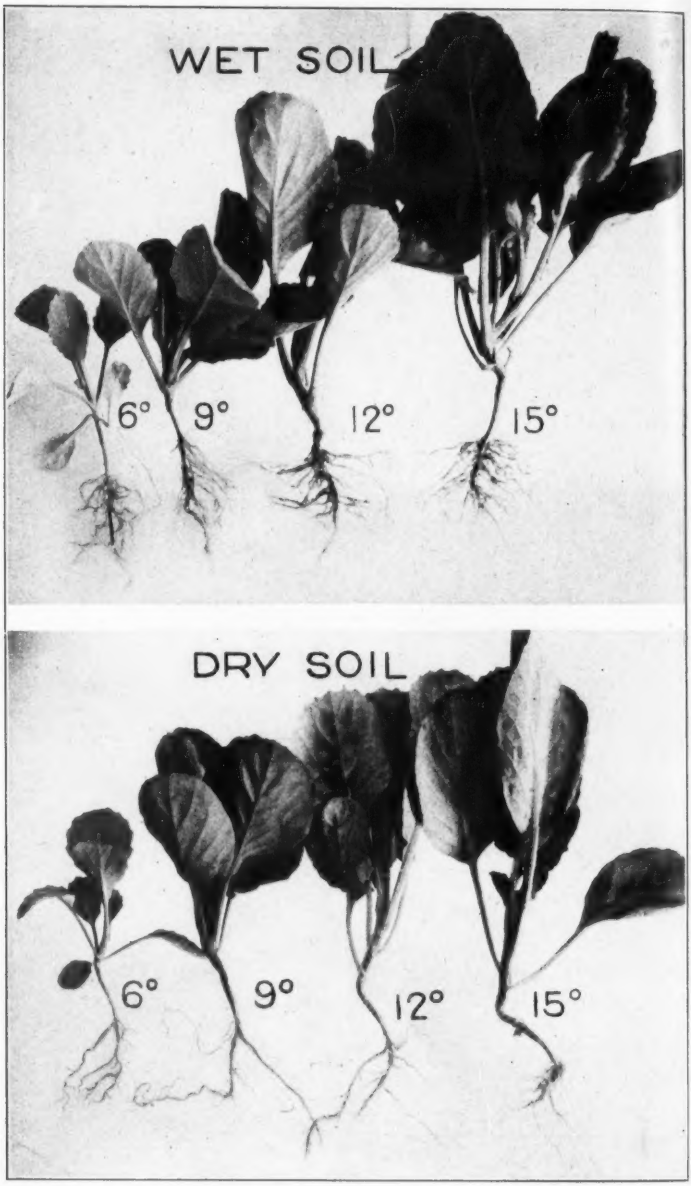

WET SOIL
6°
9°
12°
15°
DRY SOIL
6°
9°
12°
15°

PLATE 2

NFLUENCE OF SOIL TEMPERATURE AND SOIL MOISTURE UPON THE NORMAL DEVELOPMENT OF CABBAGE PLANTS

Note the progressive increase in size corresponding to rising temperatures. The plants were grown eight weeks, at the soil temperatures indicated, in a uck soil similar to soil B of text (page 552) but free from infection with the club-oot organism. The air temperature for all alike was about 15° C. Plants in he upper group were grown in soil kept uniformly at high moisture content, hat is 90 per cent of the water-holding capacity. Those below were grown in oil kept at a moisture content of 45 per cent of the water-holding capacity. When transplanted into this soil the plants were almost as large as those shown t 6° C. Very little growth occurred at that low soil temperature.

Note the somewhat larger plants grown in the soil with increased moisture.

PLATE 3

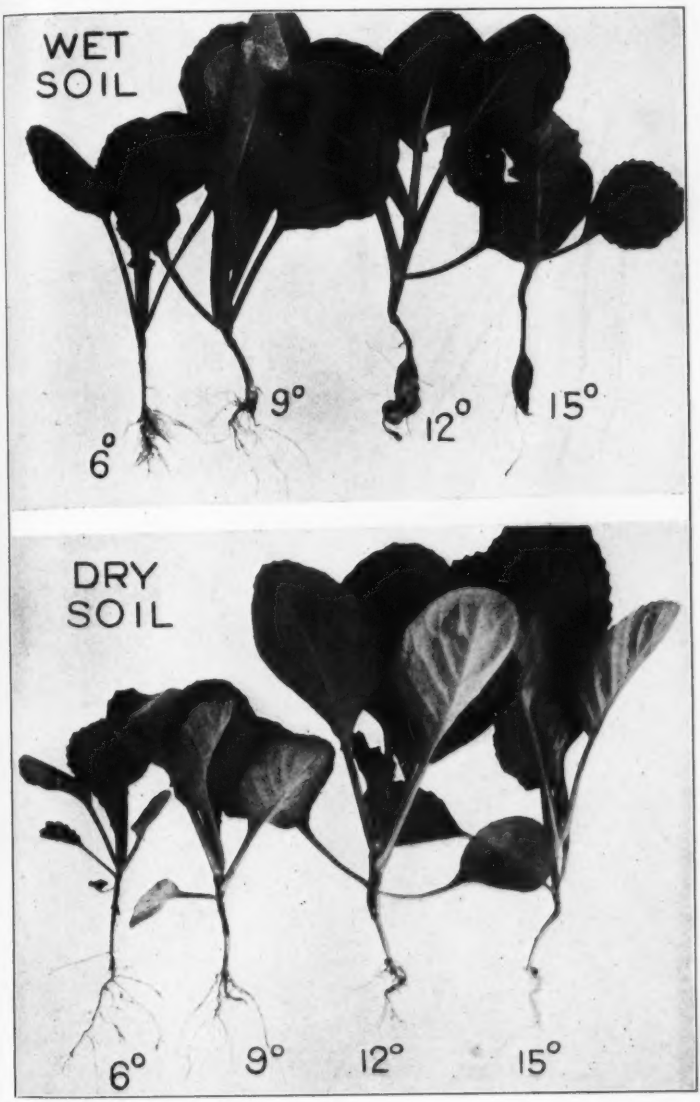
INFLUENCE OF SOIL TEMPERATURE AND SOIL MOISTURE UPON CLUBROOT OF CABBAGE

These plants correspond to those in Plate 2, but were grown in infested soil. Note that clubs occur only on roots grown in wet soil at 9°, 12°, and 15° C.

The plants were grown in soil taken from the same sample of sandy loam (soil B of text, p. 552) uniformly infested with the clubroot organism and of a nature favorable for the development of the disease. The air temperature for all alike was about 15° C.

Plants in the upper group were grown in soil kept uniformly at a high moisture content of 90 per cent of the water-holding capacity. Those below were grown in soil kept at a moisture content of 45 per cent of the water-holding capacity. The absence of clubbing in the roots from the dry soil at the three higher temperatures as compared with corresponding ones in the wet soil must therefore be due to the inhibiting influence of low soil moisture.

The influence of soil temperature on clubbing and on growth as shown in the upper group is similar to the series in soil A (Plate 1) with the exception of that grown at 9° C. In the latter case no clubbing occurred at 9°, but in this soil slight clubbing developed at 9° C.


WET
SOIL
6°
9°
12°
15°
DRY
SOIL
6°
9°
12°
15°

Effect of Soil Temperature and Soil Moisture on Clubroot

PLATE 4

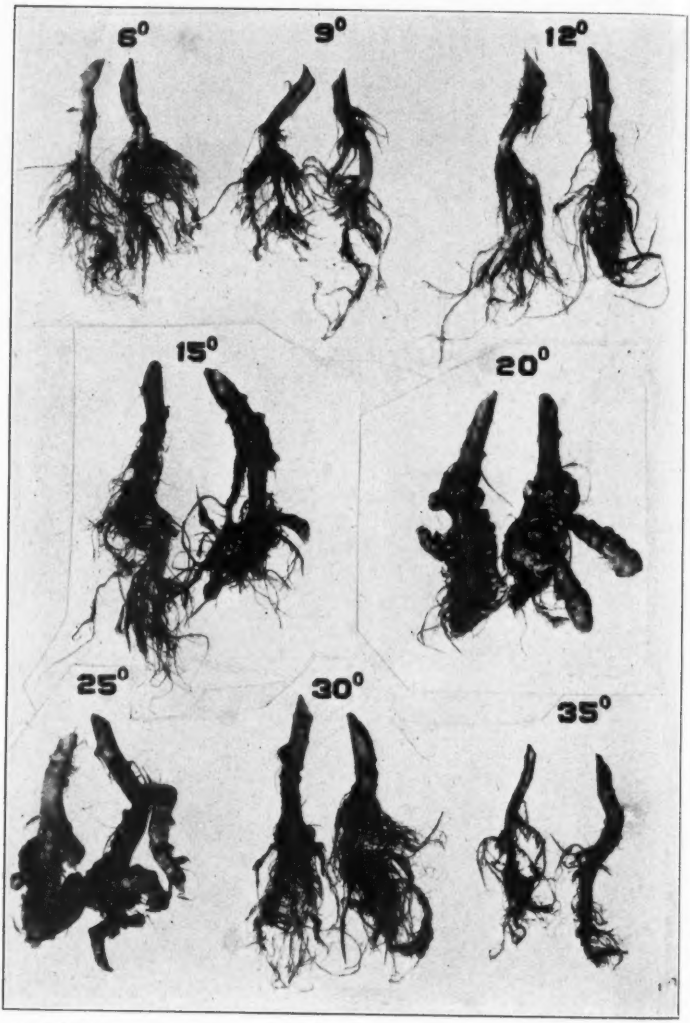


PLATE 4

INFLUENCE OF SOIL TEMPERATURE UPON CLUBROOT OF CABBAGE

Note absence of disease in the three lower (6°, 9°, 12° C.) and the highest temperature (35° C.).

Plants were grown 5 weeks, at the soil temperatures indicated, in a clay loam soil (soil A diluted with sand, see text p. 552 and 555) uniformly infested with the clubroot organism. The air temperature for all alike was about 20° C. This soil was of a nature favorable for the development of clubroot and was kept uniformly at a moisture content found favorable for the development of the disease, that is 75 per cent of the water-holding capacity.

The complete absence of clubroot in the three lower temperatures and in the highest temperature must therefore be attributed solely to the inhibiting influence of soil temperature.

Largest clubroot development at 20° C., with slightly smaller clubs at 25° C.

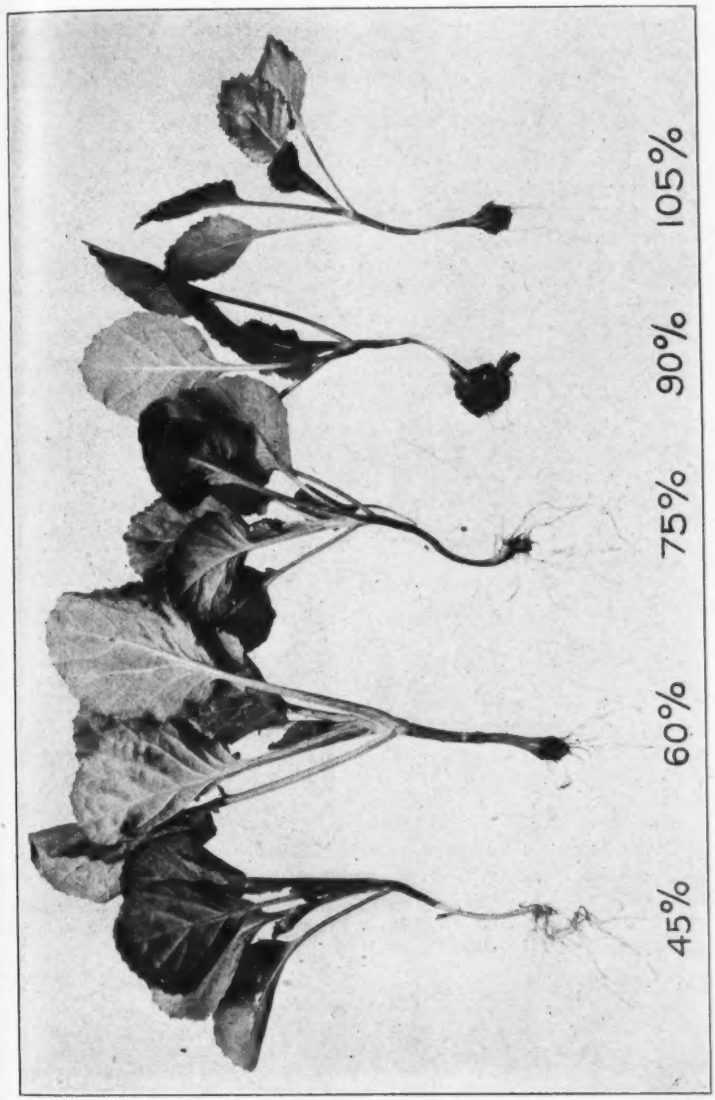
96036—24†——6

PLATE 5

INFLUENCE OF SOIL MOISTURE UPON CLUBROOT OF CABBAGE

Note absence of disease at the lower moisture, 45 per cent, and the extreme development at the highest moistures, 90 and 105 per cent.

Plants grown at like soil and air temperatures, which ranged from 12° to 18° C. Soil a sandy loam (soil B of the text, p. 552) uniformly infested with the clubroot organism and of a nature favorable for the development of clubroot. The amount of water constantly kept in the cans was equivalent to the per cent of water-holding capacity as indicated. The clubbed roots in the 75 per cent cans and above were partially decayed as a result of secondary decay organisms, so could not be removed without loss of parts of the roots that were diseased.


45%
60%
75%
90%
105%

LENGTH OF COTTON FIBER FROM BOLLS AT DIFFERENT HEIGHTS ON THE PLANT[1]

By THOMAS H. KEARNEY, *Physiologist in Charge, and* GEORGE J. HARRISON, *Field Assistant, Alkali and Drought Resistant Plant Investigations, Bureau of Plant Industry, United States Department of Agriculture*

The belief is current that in long-staple cottons fiber from the first picking is shorter than that from later pickings. This belief is probably well founded, for in 33 individual plant selections of the Yuma variety of Egyptian cotton, fiber from the second picking averaged one-sixteenth inch (1.6 mm.) longer than fiber from the first picking.[2]

The bulk of the fiber obtained from the first picking is taken from bolls on the lower fruiting branches, while the later pickings are furnished, in large part, by bolls situated higher on the plants. It is of interest, therefore, to determine the length of the fiber from bolls borne at different heights on the plant, in order to ascertain whether fiber length is correlated with the position of the boll. This paper gives the results of such determininations on the Pima variety of Egyptian cotton, grown under irrigation at the United States Field Station at Sacaton, Ariz.

Ten well-grown plants of Pima cotton were selected and a ripe boll was gathered from each successive fruiting branch on each individual. In order to determine whether these plants differed significantly in mean length of fiber, the fiber was measured on one seed from each boll collected on each individual.[3] The plant means as thus determined are given in Table I.

TABLE I.—*Mean fiber length of the 10 plants of Pima cotton used in determining the relation between height of fruiting branch and length of fiber*

Plant No.	Number of determinations	Mean length of fiber	Plant No.	Number of determinations	Mean length of fiber
		Millimeters			*Millimeters*
1	12	40.7±0.57	6	12	41.0±0.31
2	13	41.9± .66	7	12	43.1± .94
3	13	41.0± .31	8	11	41.9± .40
4	13	42.5± .75	9	12	40.7± .67
5	11	41.6± .41	10	11	41.9± .30

The individual plants show a range in mean fiber length amounting to 2.4 mm. but the difference between the plants having the longest and shortest fiber, respectively, is not significant, being only slightly more than twice its probable error. In computing the mean length of fiber for successive heights on the plant, it was therefore considered proper to take the bolls from all 10 plants as one array.

[1] Received for publication April 9, 1924.

[2] KEARNEY, T. H. FIBER FROM DIFFERENT PICKINGS OF EGYPTIAN COTTON. U. S. Dept. Agr., Bur Plant Indus. Circ. 110: 37-39. 1913.

[3] For a description of the method used in determining length of fiber see: KEARNEY, T. H. SEGREGATION AND CORRELATION OF CHARACTERS IN AN UPLAND-EGYPTIAN COTTON HYBRID. U. S. Dept. Agr. Bul. 1164, p. 10. 1923.

In order to smooth the curve and reduce the probable errors of the means, they were computed for each successive three fruiting branches, taken as one array. Fruiting branches in Pima cotton are rarely retained at nodes lower than No. 9 and the numbers of bolls which matured on fruiting branches higher than No. 32 were too small to give reliable data. The mean fiber lengths for the 8 groups of fruiting branches between nodes 9 and 32, inclusive, are stated in Table II and the data are presented graphically in figure 1.

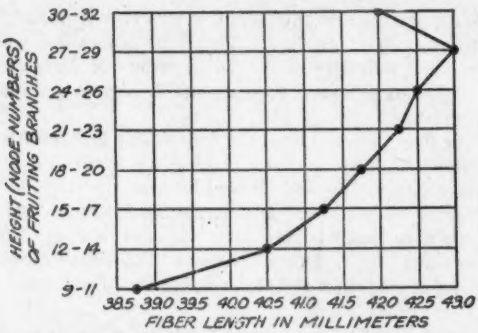


FIG. 1.—Average length in millimeters of Pima cotton fiber from bolls borne at successive heights on the plant, the heights being indicated by the numbers of the nodes of the main stalk at which the fruiting branches are borne.

TABLE II.—*Mean length of Pima cotton fiber from bolls borne at successive heights on the plant, the heights being indicated by the numbers of the nodes of the main stalk at which the fruiting branches are borne*

Node numbers of the fruiting branches	Number of determinations	Mean length of fiber	Node numbers of the fruiting branches	Number of determinations	Mean length of fiber
	Bolls [a]	*Millimeters*		*Bolls* [a]	*Millimeters*
9–11	10	38.75±0.85	21–23	10	42.20±0.45
12–14	19	40.46± .36	24–26	19	42.41± .25
15–17	11	41.27± .31	27–29	12	42.89± .40
18–20	20	41.75± .40	30–32	17	42.05± .40

[a] The absence of mature bolls on many of the fruiting branches at the time the collection was made accounts for the fact that the number of determinations is in all cases fewer than 30 (10 plants x 3 fruiting branches).

Although the differences in mean fiber length as between any two successive groups of fruiting branches, are in no case significant, there is a steady increase from the lowest to the next to the highest group. The highest group (nodes 30 to 32) shows a slight decrease in length of fiber as compared with the group just below (nodes 27 to 29).

The fiber in the bolls borne on fruiting branches at nodes 9 to 14, from which a large part of the first picking probably is derived, is decidedly shorter than the fiber produced higher on the plant. Comparing the lower half of the fruiting zone (nodes 9 to 20) with the upper half (nodes 21 to 32) the means for fiber length are 40.75±0.28 and 42.37±0.19, respectively, the difference being 1.62± 0.34. This difference evidently is significant, being nearly 5 times its probable error.

It may be concluded, therefore, that bolls borne on the lower fruiting branches, constituting the so-called "bottom crop," produce shorter fiber than bolls that are situated higher on the plant.

Another series of determinations of fiber length was made on bolls from flowers which had opened in successive periods during July and August, 1921, the object having been to ascertain whether there is a consistent relation between the length of the fiber and the date of opening of the flower from which the boll developed, regardless of the height on the plant of the fruiting branch on which the boll was borne. In this case also fiber was measured on one seed from each boll.[4] The data obtained are given in Table III.

TABLE III.—*Mean length of Pima cotton fiber in bolls from flowers which had opened during successive periods in 1921*

Dates of flowering	Mean length of fiber	Dates of flowering	Mean length of fiber
	Millimeters.		*Millimeters*
July 22 to 24	39.59±0.33	Aug. 14 and 15	41.09±0.32
July 27 to 30	41.90± .20	Aug. 23 and 26	41.72± .29
Aug. 8 to 10	41.36± .33		

The fiber from flowers opening during the first period (July 22 to 24) is significantly shorter than that from any of the later flowers but there are no significant differences in fiber length among any of the later periods. It is probable that the flowers which opened during the period July 22 to 24 were borne, for the most part, on low fruiting branches, while flowers which opened later may have been borne either on higher branches or on nodes farther out on the lower fruiting branches.[5]

While generalization from such limited data is unsafe, these results, as compared with those given in Table II, point to the conclusion that the length of fiber is affected less by the date of flowering than by the height on the plant of the fruiting branch on which the boll is borne.

[4] Through an oversight, no record was made of the numbers of determinations on which are based the means for each period.

[5] W. Lawrence Balls studied in Egypt the relation between date of flowering and length of fiber, tagging 20 flowers daily from July 7 to September 1. His data (BALLS, W. L. THE DEVELOPMENT AND PROPERTIES OF RAW COTTON, p. 198–203, Tables I–II; p. 90, fig. 4, London, 1915) show marked fluctuation during the flowering season, the minimum length of fiber having been reached in bolls from flowers opening during the 5-day period July 27–31 and the maximum length in bolls from flowers opening during the 5-day period August 9–13. The difference in fiber length as between the two periods amounted to 2.9 mm.

"HAIRY NECK" WHEAT SEGREGATES FROM WHEAT-RYE HYBRIDS[1]

By CLYDE E. LEIGHTY, *Agronomist in Charge of Eastern Wheat Investigations, and* J. W. TAYLOR, *Assistant Agronomist, Office of Cereal Investigations, Bureau of Plant Industry, United States Department of Agriculture*

Pollen from rye was successfully used to fertilize wheat flowers as early as 1875 by Wilson (*14*)[2] in Scotland. Subsequently many plant breeders, both in Europe and America, have made this cross and produced F_1 plants. The natural wheat-rye hybrid has been observed frequently in wheat plats, particularly at the Arlington Experiment Farm of the United States Department of Agriculture, Rosslyn, Va. (*8*) (see Pl. 1), and at Saratov, Russia (*9*). However, despite the many F_1 plants produced as the result of these artificial or natural cross-pollinations, few studies on the inheritance of specific morphological characters for other than the F_1 generation have been made and very few fixed segregates having any resemblance to rye have been reported. The plants described in this paper are believed, therefore, to be of peculiar interest in that they are like wheat, except for a single undoubted rye character, and in some cases have produced progeny all of which were like themselves.

SELF-STERILITY IN WHEAT-RYE HYBRIDS

The cross between wheat and rye is intergeneric and the F_1 hybrid is either entirely sterile or only slightly fertile. As shown by Leighty (*8*), several experimenters have reported a small percentage of fertility in this generation. One natural hybrid found by Leighty had a fertility of 5 per cent, which is apparently unusually high. When the F_1 shows fertility and no precautions have been taken to prevent natural backcrossing, there is always the probability that the seeds present are due to the effect of wind-borne pollen of wheat or rye. The writers have no evidence that viable pollen is ever formed by the F_1 plants, and, furthermore, they have never observed opening of the anther sacs. Jesenko (*4*) found the F_1 wheat-rye hybrid self-sterile but slightly fertile with wheat pollen and very rarely so with pollen of rye. Investigations by the writers indicate that fertility in the F_1 nearly always is due to backcrossing with wheat pollen.

At Arlington Experiment Farm, during the crop seasons of 1922 and 1923, over 8,000 flowers of natural F_1 wheat-rye hybrids growing in the wheat plats were bagged with glassine bags before blooming and approximately 16,000 flowers were unprotected. The results, given in Table I, indicate that the F_1 was self-sterile, but slightly fertile when open-pollinated. Therefore, unless the pollination of the F_1 wheat-rye hybrid is controlled the source of pollen will be in doubt and inheritance can not be determined with accuracy.

[1] Received for publication April 1, 1924.

[2] Reference is made by number (italic) to "Literature cited," p. 575-576.

TABLE I.—*Number of heads and flowers, bagged and not bagged, on F_1 wheat-rye hybrid plants growing in wheat plats and number and percentage of seed set on these heads*

Year.	Number of heads		Number of flowers		Number and percentage of seed set on heads		
	Bagged	Not bagged	Bagged	Not bagged	Bagged	Not bagged	
						Total	*Per cent*
1922	142	240	6,972	11,791	[a] 1	77	0.65
1923	34	69	1,374	4,772	0	32	.67
Total	176	309	8,346	16,563	1	109	.66

[a] The single seed obtained under bagged conditions is likely due to late or faulty bagging.

In this connection it should be explained that the rye varieties or selections are grown at the Arlington Experiment Farm in twentieth-acre plats, separated by several wheat plats in such a manner that the rye varieties are approximately 90 feet from one another. This tends to reduce somewhat the amount of natural crossing between the rye varieties, but furnishes abundant opportunities for natural crossing between the wheat and rye. Many natural hybrids between these have been found on this farm since 1914, the year in which the first one was discovered (*6*).

In 1918, 40 seeds were obtained from 19 natural F_1 wheat-rye hybrids found in the wheat plats at Arlington (*8*). The pollination of these plants was uncontrolled, and it is likely that the F_2 generation plants were natural sesqui-hybrids. The F_2 and F_3 of the wheat-rye hybrids were grown in the greenhouse, where natural crossing with wheat was again possible, though not to the degree existing under field conditions. The later generations were matured in the field rod-row nursery in order to test the yielding ability of the selections. The generation grown in 1922 consisted of 77 rod-rows, or approximately 9,400 plants. This generation was made up of about 63 strains, descended from 24 F_3 plants. These strains, in general, were fully as fertile in the F_5 generation as wheat grown under the same conditions. High fertility in these wheat-rye hybrids was first observed in certain plants of the F_3 generation.

The head type of the F_5 generation plants, grown in the 77 rod-rows, was for the most part identical with that of wheat, and only rarely was a plant found which differed from wheat to a degree that approached the F_1. The majority of the plants could not be distinguished from common wheat. One undoubted rye character, however, was observed on eight plants which in other respects could be called wheat. This was the pubescent peduncle commonly associated with rye, but apparently unknown in wheat (Pl. 2).

THE "HAIRY-NECK" CHARACTER

The peduncle is the upper portion of the stem (culm) and in the cereals is usually considered as being the uppermost internode at the apical node of which the head or spike is attached. The portion of the peduncle just below the head is often called the "neck," and when this is pubescent or covered with hairs, is here referred to as a "hairy neck."

The peduncle of rye is more slender than that of wheat, and its upper portion, the neck, for a distance of a fraction of an inch to three or more inches below the head, is usually covered with short hairs readily discernible to the naked eye. The lower segments of the rachis are also pubescent, but only the neck character is here considered. In some cases the hairiness, located around the apical node,

is limited to a few hairs which are difficult to distinguish without magnification. Rye plants with glabrous peduncles are sometimes found. The percentage of such plants is usually very small, but there apparently are varietal differences in regard to this factor despite the fact that the rye plant is almost entirely cross-pollinated. The necks of four typical rye heads are shown in Plate 2.

In Table II are given the data obtained on eight varieties of winter rye for the crop year 1922–23, in regard to the hairiness of the neck as seen by the naked eye. In five of the eight varieties, less than one per cent of glabrous-necked culms were found, while in von Rümker No. 2 there were 8 per cent. The total for all varieties was 1.9 per cent glabrous-necked culms. Gaines and Stevenson (*3*) report 66 per cent hairy-necked plants among 41 plants of Rosen rye examined at Pullman, Wash.

TABLE II.—*Number and per cent of glabrous-necked culms found in eight varieties of winter rye at Arlington Experiment Farm in 1923*

Variety	Number of culms examined	Glabrous-necked culms		Variety	Number of culms examined	Glabrous-necked culms	
		Number	Percentage			Number	Percentage
Von Rümker No. 2	500	40	8.0	Winter	638	5	0.8
St. Johns	484	7	1.4	Abruzzes	643	4	.6
Rosen	1,363	45	3.3	Von Rümker No. 1	700	4	.6
Virginia	490	3	.6				
Rimpau	480	4	.8	Total	6,587	128	1.9
Mexican	1,289	16	1.2				

The F_1 wheat-rye hybrid is approximately intermediate between the two parents in such characters as shape and density of head, number of spikelets, width and other characters of the empty glumes, and in general appearance (Pl. 1). The hairy-neck character of rye, however, is usually expressed in the F_1 and apparently is a dominant character in this generation. About 300 first generation wheat-rye hybrid plants, nearly all natural hybrids, growing at the Arlington Experiment Farm, have been examined with reference to this character. Approximately 80 per cent of these have had the hairy neck in the varying degrees as found in rye. The glabrous-necked plants in the F_1 may be due to the gametic constitution of the rye parents in these cases in respect to this character.

INVESTIGATIONS ON HAIRY NECK

Plants resembling wheat in most characters, but having the hairy neck, were observed in the F_2 and subsequent generations arising from the natural hybrids found in 1918. Investigations on this character were begun in 1920, when a cross was made between Purplestraw wheat and a wheat-like F_3 segregate of a wheat-rye hybrid. The pollen parent of this hybrid, the F_3 segregate, apparently carried the factor for hairy neck in heterozygous condition, for, from the 8 kernels resulting from this cross, 7 glabrous-necked plants were produced and only 1 with hairy neck. The progeny of this hairy-necked plant in 1922 consisted of 36 glabrous-necked plants and 2 with hairy necks. Data on the progeny of these 2 hairy-necked plants obtained in 1923, are given in Table III (A and B). In addition to these 2 plants, 8 other plants having the hairy neck were selected in 1922 from the F_3 progeny of the natural wheat-rye hybrids found in 1918. Data on the plants produced in 1923 from the seed of these 8 plants are given in Table III (C to K), and 3 of the heads, together with a head of Fulcaster wheat for comparison, are shown in Plate 3.

TABLE III.—*Total number of plants and numbers with hairy neck and glabrous neck in the progeny of each of 10 wheat-like plants with hairy necks selected in the progeny of wheat-rye hybrids, with data on certain head characters*

Selection number and head characters	Number of progeny plants				Head characters of progeny
	Total	Hairy-necked		Glabrous-necked	
		Number	*Per cent*		
Awnless, white-chaffed:					
A	92	13	14	79	(a)
B	100	16	16	84	(a)
C	40	40	100	0	(a)
D	28	9	32	19	(b)
E	66	5	8	61	(b)
F	59	13	22	46	(a)
Awned, white-chaffed:					
G	26	13	50	13	(b)
H	18	18	100	0	(a)
I	31	22	71	9	(b)
K	98	98	100	0	(c)

(*a*) All plants with the same head characters as the parent selection.
(*b*) One semiawned plant with red chaff included, all others with the same head characters as the parent selection.
(*c*) Eighty-four bearded, white-chaffed plants, 14 semiawned plants, all white-chaffed.

All 10 of the plants selected in 1922 had been fertilized naturally, no attempt being made to control pollination by bagging or isolation. These plants were fully fertile, with the exception of selection K, in which the fertility was about 75 per cent. The seed of these plants germinated well and about 75 per cent of the kernels produced plants the next year, which was fully equal to the performance of several pure lines of wheat sown in the same nursery.

The pubescence on the peduncles of the several selections made in 1922 varied somewhat in amount and extent. Several of the necks are shown in Plate 4, together with the necks of wheat and rye. The pubescence on selection I is very heavy, the hairs extending down the neck for 5 or more inches below the head, which is farther than has been observed on any rye plant examined. Selection K has a sparse pubescence, extending down the neck not more than an inch. The pubescence in the progenies of different selections in some cases was of much the same type as that of the parental selection, while in other cases different members of the progeny of a selected parent varied considerably among themselves as to pubescence.

In the progenies of the 10 selections, as shown in Table III, there were in all 247 plants with hairy necks and 311 with glabrous necks. Only 3 of these 10 selections, C, H, and K, produced plants all of which were hairy-necked. It is probable that these progenies are homozygous with respect to this character, but this is not regarded as a certainty. The expression of the character in later generations must be observed before it can be concluded that strains entirely homozygous in respect to the hairy-neck character have been established.

On the matter of the fixation of the hairy-neck character, Carman (*1*) who conducted some of the earliest and most extensive experiments on wheat-rye hybrids, reports as follows:

> The Rural New Yorker No. 6 is one of the rye-wheat hybrids, though all appearance of rye has disappeared except that the culms just under the heads are now and again downy as in rye. This downiness of the stem is variable. We have tried by selection for many years to fix it without any approach to success. Of all our rye-wheat hybrids, the downy culm is permanent in but one, and that resembles rye in several other respects.

The progenies of the other 7 selections (A, B, D, E, F, G, and I) consisted of both hairy-necked and glabrous-necked plants, there being a total of 91 plants with hairy necks and 311 with glabrous necks, or very nearly a 1 to 3 ratio.

This is the ratio that would be expected if the hairy-neck character is recessive. But in the different progenies the ratios of hairy-necked to glabrous-necked plants varied from 1 to 12.2 in selection E to 2.4 to 1 in selection I. Furthermore, all of the parent plants had hairy necks. Therefore, the character does not appear to be recessive.

The hairy-neck character, however, has not been consistently dominant, as is evident from data given above. Nevertheless, at least a high degree of dominance occurs in the F_1. Additional data on the F_2 generation of wheat-rye hybrids also are at hand. One lot of such plants grown by the writers in 1923 consisted of 21 with hairy necks and 8 with glabrous necks. These were descended from 24 natural hybrids, all with hairy necks, and all open-fertilized. Gaines and Stevenson (*3*) report that in the F_2 they obtained 11 plants with hairy necks and 4 with glabrous necks.

POSSIBILITY OF NATURAL CROSSING

It is known that natural crossing occurs to some extent in wheat-rye hybrids. The data presented in Table I indicate that fertility in the F_1 is dependent on pollen from outside sources and not on self-pollination. The plants used in this study of the hairy-neck character were open-fertilized throughout the experiment, as were also all parental plants in preceding generations. In 1923, however, heads to be used in further studies were selfed by bagging. In this connection it should be noted that the percentage of fertility of 21 bagged (selfed) heads, excepting 2 in which the fertility for some reason was low, was 77 per cent, while the percentage of fertility of 21 unbagged (open-fertilized) heads, was 79 per cent. Some of the selected heads, both selfed amd open-fertilized, showed higher fertility than Purplestraw wheat, which varied from 80 to 85 per cent, but others were somewhat less fertile.

The progenies of the selections listed in Table III do not disclose a large amount of natural crossing. All plants in 5 of the 10 progenies were identical with their respective parents in head characters. Four of the progenies each contained one individual that appeared from its head characters to be due to a natural cross on the parent selection, the off-type plant in each case differing from the parental selection in degree of awn development and in glume color.

Considerable natural crossing was evident in only one progeny (K), which consisted of 84 awned and 14 semiawned plants. These plants lacking full development of awns, in the progeny of an awned plant, indicate natural crossing with pollen from an awnless plant, inasmuch as the factor for presence of awns in wheat normally is incompletely recessive to the factor for their absence, which results in a semiawned plant in the F_1. In spite of this evidence of natural crossing all plants in selection K were hairy-necked. There is perhaps a slight possibility that the different head characters appearing in this progeny, likewise also in the other cases noted, are not evidence of natural crossing but indicate some unusual factorial composition of the gametes, resulting in abnormal segregation.

In addition to the differences in respect to awns, the progeny of selection K, though consisting entirely of hairy-necked individuals, also differed somewhat in head type, as is shown in Plate 5. All heads had white or yellow glumes, but one type had a lax, fusiform spike, while that of the other was more dense and oblong in shape. The width and shape of the shoulder of the outer glume, as well as the length of the beak, differed greatly, as may be seen from the figure.

In Plate 2 are shown the kernels obtained from one head each of Abruzzes rye, Fulcaster wheat, and the fusiform and oblong types of head present in selection K. Resemblance to rye may be seen in the seeds from the fusiform head. The

kernels are narrower, blunter at the base, and have longer and more sharply pointed germs than the kernels from the oblong head. The longer, narrower glume of the fusiform head no doubt influences the shape of the grain, but whether or not the glume character can be attributed to the F_1 rye parent is open to question. According to Fruwirth (*2*, *p. 183–184*), a segregate somewhat similar to this fusiform type resulted from a wheat-rye hybrid made by Rimpau in 1888. It was largely wheat-like, but in comparison to wheat the head and glumes were longer, the latter closely pressed together, and the keel ciliated as in rye. This strain was grown for many years in an experimental way in Europe and remained fully constant in all respects, including a partial sterility exhibited by it.

DISCUSSION AND SUMMARY

It is evident that the hairy neck found in wheat-like segregates of wheat-rye hybrids is a heritable character, but the data at hand are not sufficient to permit definite conclusions regarding the number of factors involved or the manner in which these factors are transmitted. Considerable irregularity in segregation is apparent and both dominant and recessive tendencies are observed. The reason for this is unknown, but it is suspected that it is related to probable irregularities in chromosome behavior.

According to Sakamura (*11*), *Triticum vulgare* has 21 and *Secale cereale* 7 haploid chromosomes. These numbers have been confirmed by Sax (*12*). The number of chromosomes in the F_1 of the hybrid between these species has not been determined. Nakao (*10*), who made cytological studies of the F_1 of a wheat-rye hybrid, states that "the chromosome number is not definite (17–23), being almost always more than the expected number, 16." [8] From our present knowledge of chromosome numbers in these species as given by Sakamura (*11*) and Sax (*12*), we would expect 28 chromosomes in the F_1, 21 from the *Triticum vulgare* parent, and 7 from the *Secale cereale*. This would be in accord with the observation of Kihara (*5*), who found 35 somatic chromosomes in the F_1 of hybrids between different members of the "Emmer group" of wheat, which contain 14 gametic chromosomes, and members of the "Vulgare group" of wheat, which contain 21 gametic chromosomes. Sax (*12*) also observed 35 chromosomes in hybrids between these groups, while in an F_1 hybrid between *T. monococcum* with 7 gametic chromosomes and *T. turgidum* with 14, the F_1 had 21 somatic chromosomes. In the F_1 hybrid between the "Emmer" and "Vulgare" groups, according to both Kihara and Sax, 14 pairs of chromosomes and 7 univalent chromosomes are found in the pollen mother cell. The 14 pairs, as stated by Sax, "divide as usual in meiosis while the 7 univalents lag behind but ultimately divide equationally in the first division, and pass at random, without dividing, to either pole in the homoeotypic division." As a result of these abnormalities, Sax estimates that 20 per cent of the pollen produced is obviously imperfect and a large percentage is nonfunctional. Considerable sterility is found in these hybrids between the groups of wheat.

If such phenomena should occur in the wheat-rye hybrid there would be 7 bivalent and 14 univalent chromosomes in the F_1 generation. With the higher number of univalents and the increased ratio of these to bivalents, abnormalities in chromosome behavior would be expected to occur in even greater degree than in the hybrids between different groups of wheat. Furthermore, with the gametes effecting the hybrid having come from plants belonging to different genera, incompatibility between the chromosomes contributed by the respective parents could hardly fail to exist. The results of Nakao (*10*) confirm this expecta-

[8] Nakao states: "In wheat, as described by Overton and Koernicke, and in rye, the reduced number of chromosomes is 8."

tion. In his studies on the nuclear divisions of the pollen mother cells of the wheat-rye hybrid he found degenerative phenomena in the pollen mother cell and in the pollen. "The degenerating phenomena of the pollen cells are observed at every stage of their development, e. g., in synapsis, spireme, the first division, the second division, etc." Jesenko (*4*) in studies of the pollen mother cell of the wheat-rye hybrid also found abnormalities which would seldom permit the formation of fertile pollen. These cells seldom divided into 4 parts, as is natural in wheat and rye, but frequently divided into 3, 5, 6, or even more different parts.

Pollen grains are formed in the F_1 wheat-rye hybrid, but nearly all of them are shrunken and poorly developed, and lack the usual cell contents. A few are found now and then, however, that are better developed and that may be capable of growth. But anthers filled with such pollen have never been found, and, probably on account of the resulting lack of internal pressure, no anther has been observed to dehisce. F_1 plants isolated or bagged have always been sterile. Pollinations made by artificially rupturing the anthers also have not been successful. Jesenko pollinated over 3,000 hybrid flowers with pollen forced out of the anthers but no flower set seed. The writers have made several hundred such pollinations with like result.

The F_1 wheat-rye hybrid appears to be self-sterile, but seeds sometimes are set on such plants when open pollinated. In 1922, at the Arlington Experiment Farm, 11,791 wind-pollinated flowers of F_1 wheat-rye hybrids growing in the wheat plats produced 6.5 seeds per thousand, and in 1923, under similar conditions, 4,772 hybrid flowers produced 6.7 seeds per thousand. The results for the two years agree so closely that it appears that heredity rather than environment is the factor determining seed formation. Leighty and Hutcheson (*7*) found, in 1919, that of the wheat flowers emasculated and exposed in the field at Arlington, 83.3 per cent set seed, a high seed formation for wheat heads. This indicates that an abundance of wheat pollen was disseminated by the wind to effect fertilization of exposed stigmas. It is well known also that rye pollen is widely disseminated by the wind. The flowers of the wheat-rye hybrid open at an average date intermediate between the average for wheat and rye, and, as is usual with small grain plants when fertilization has not occurred, remain open for a week or longer. Since the natural wheat-rye hybrids at Arlington grew in close proximity to both wheat and rye plants, there is little doubt that all their flowers were pollinated at some time or other with wind-borne pollen of both wheat and rye. The flowers thus pollinated produced on the average about 6.6 seeds per thousand. Jesenko (*4*) obtained 3 seeds per thousand pollinations when he backcrossed his F_1 wheat-rye hybrid with wheat pollen (and one seed from almost 5,000 pollinations with rye). The difference in results obtained by the writers and by Jesenko probably indicates the greater effectiveness of natural pollination, and is in line with the results usually obtained in artificial crosses of wheat.

Although the F_1 wheat-rye hybrid has produced no seed when bagged, but when open pollinated has shown 0.66 per cent of fertility, it cannot be definitely concluded that the female gamete is more fertile than the male. No cytological studies of the egg mother cell of the F_1 wheat-rye hybrid have been made and nothing is known regarding the phenomena connected with development of the female gamete. Self-sterility, when a head is bagged, may be due to lack of development of sufficient pollen in the anther to cause dehiscence. Following artifical rupture of the anthers and self pollination, it may be due to the same causes that are responsible for the usually nearly complete self-sterility in rye itself. It is evident, however, that the stigmas of the F_1 are brought into contact with viable pollen of wheat and rye more frequently than the pollen of the

F_1 is brought into contact with stigmas of any kind, and especially with ovules capable of being fertilized. Thus, the apparent greater fertility of the female gamete may be due merely to chance.

With 7 bivalent and 14 univalent chromosomes present in the F_1, numerous recombinations at the time of gamete formation would be theoretically possible. It is probable that the gametes formed would contain 7 bivalents in every case and that nearly all of the gametes would contain, in addition, one or more of the univalents. Assuming random assortment, one gamete out of 16,384, on the average, would be expected to contain no univalent, and an equal number would contain all 14 univalents.

There is some basis for assuming that successful backcrosses on the F_1 wheat-rye hybrid with wheat and rye pollen are related in some way to the number of univalents present in the hybrid gamete, and that with a high number of univalents a successful backcross with wheat can result, while with none or possibly one univalent the rye backcross is possible. If it is assumed that a gamete with 12, 13, or 14 univalents is required for a successful backcross with wheat pollen the theory would explain the results obtained. The 12, 13, or 14 univalents present in the gamete would appear to counteract any incompatibility that may exist between wheat and rye chromosomes to a sufficient degree to allow the setting of seed.

Sakamura (*11*) found 42 somatic chromosomes in a fertile strain descended from a backcrossed wheat-rye hybrid. Kihara (*5*) confirmed this number in the fifth generation of this same lot of fertile hybrids. Two plants resulting from seed produced by an almost sterile plant in this same lot of wheat-rye hybrid descendants contained 38 and 42 somatic chromosomes, respectively.

Jesenko (*4*) observed a self-fertile plant with dense hairiness on the sheaths of the lower leaves in the progeny of an F_1 wheat-rye hybrid backcrossed with wheat. This character was not observed in either the wheat or rye parent. In the F_3 generation of this hairy-sheathed plant there were 31 hairy-sheathed individuals and 23 glabrous-sheathed, or approximately a 9 : 7 ratio. On the assumption that 2 factors are concerned in the inheritance, there should be 2 equal groups in the F_4, segregating into 3 : 1 and 9 : 7 ratios, or a total of 3 hairy plants to 2 glabrous plants. The actual ratio obtained by Jesenko was 3 : 1.96. This is apparently an example of simple Mendelian inheritance in wheat-rye hybrids.

The unusual ratios obtained on the segregation for the hairy-neck character can not be explained satisfactorily, as they are based upon too few plants, and also upon plants in the ancestry of which natural crossing has occurred at least to a small extent. Further investigations may show that we are observing plants in which an excess of recessive factors, accumulated in the gametes through random assortment of wheat and rye chromosomes, results at times in a reversal of dominance, and at times in unusual ratios in inheritance. If this is the case it is possible that the three selections showing all hairy-necked individuals in their progeny are not fixed for this character, and that it may be impossible of fixation.

The F_2 and later generations of wheat-rye hybrids show greater fertility but less rye resemblance than the F_1. As the F_2 is usually a sesqui-hybrid with the parentage (*T. vulgare* ♀ × *S. cereale* ♂) ♀ × *T. vulgare* ♂ (21 haploid chromosomes × 7 haploid chromosomes) × 21 haploid chromosomes, the rapid disappearance of the rye characters in generations later than the F_1 appears explainable. In the F_1 generation two complete sets of chromosomes are present, one from the wheat parent, the other from the rye. The possibility of obtaining, in the F_1, an egg cell containing only 7 rye chromosomes or 21 wheat chromosomes, is extremely

small,[4] but if such occurred and fertilization were effected with a pollen grain from wheat or rye, respectively, the F_1 type would be expected in the following generation. This has been seldom, if ever, realized. Individuals with certain rye characters and others showing little rye influence morphologically but exhibiting a high degree of sterility are to be found, however, in all generations so far grown, though the number of such individuals diminishes annually. The increasing resemblance to wheat and the increasing fertility is supposed to be accompanied by progressive change in the chromosome number and constitution in the direction of the wheat type. This would be comparable to the phenomena observed by Sax (*13*) in hybrids between wheat groups with different chromosome numbers.

The hairy-neck character dealt with in this paper is an example of a definite rye character present in individuals of the F_6 generation which may be traced to the original rye parent, the parent with the lesser number of chromosomes. In other respects the selections possessing the hairy necks appear similar to wheat, and exhibit very little sterility. This is of interest and possibly of importance, inasmuch as the result obtained in interspecific wheat crosses, made with the aim of combining desirable characters of two species, have been disappointing for the most part, as the segregates reverted to the parental types, seldom showing recombinations of characters of both parents. The previously reported segregates of wheat-rye hybrids nearly always reverted rapidly to wheat, but it appears from the results reported above that it is possible to obtain plants combining certain characters of the wheat and rye parents.

LITERATURE CITED

(1) [Carman, E. S.]
1894. ruralisms. [Two Rural New Yorker Wheats, no. 57 and no. 6.] Rural New Yorker 53: 634–635, illus.

(2) Fruwirth, C.
1910. die züchtung der landwirtschaftlichen kulturpflanzen. Aufl. 2, Bd. 4. Berlin.

(3) Gaines, E. F., and Stevenson, F. J.
1922. rye-wheat and wheat-rye hybrids. Jour. Heredity 13: 81–90, illus.

(4) Jesenko, F.
1913. über getreide-speziesbastarde (weizen-roggen). Ztschr. Induk. Abstam. u. Vererb. 10: 311–326, illus.

(5) Kihara, H.
1919. über cytologische studien bei einigen getreidearten. mitteilung i. spezies-bastarde des weizens und weizen-roggen-bastard. Bot. Mag. Tokyo 33: 17–38.

(6) Leighty, C. E.
1915. natural wheat-rye hybrids. Jour. Amer. Soc. Agron. 7: 209–216, illus.

(7) ——— and Hutcheson, T. B.
1919. on the blooming and fertilization of wheat flowers. Jour. Amer. Soc. Agron. 11: 143–162.

(8) ———
1920. natural wheat-rye hybrids of 1918. Jour. Heredity 11: 129–136, illus.

[4] Once each in over 2 million gametes.

(9) MEISTER, G. K.
1921. NATURAL HYBRIDIZATION OF WHEAT AND RYE IN RUSSIA. Jour. Heredity 12: 467–470.

(10) NAKAO, M.
1911. CYTOLOGICAL STUDIES ON THE NUCLEAR DIVISION OF THE POLLEN MOTHER-CELLS OF SOME CEREALS AND THEIR HYBRIDS. Jour. Col. Agr. Tohoku Imp. Univ. Sapporo, Japan 4: 173–190, illus.

(11) SAKAMURA, T.
1918. KURZE MITTEILUNG ÜBER DIE CHROMOSOMENZAHLEN UND DIE VERWANDTSCHAFTSVERHÄLTNISSE DER TRITICUM-ARTEN. Bot. Mag. Tokyo 32: 151–154.

(12) SAX, K.
1922. STERILITY IN WHEAT HYBRIDS. II. CHROMOSOME BEHAVIOR IN PARTIALLY STERILE HYBRIDS. Genetics 7: 513–552, illus.

(13) ———
1923. THE RELATION BETWEEN CHROMOSOME NUMBER, MORPHOLOGICAL CHARACTERS AND RUST RESISTANCE IN SEGREGATES OF PARTIALLY STERILE WHEAT HYBRIDS. Genetics 8: 301–321, illus.

(14) WILSON, A. S.
1876. WHEAT AND RYE HYBRIDS. Trans. and Proc. Bot. Soc. Edinb. 12: 286–288.

PLATE 1

Heads of Purplestraw wheat (A), rye (D) and two natural F_1 wheat-rye hybrids, one with pubescent or hairy neck (B), and one glabrous neck (C). (Natural size.)

A
B
C
D

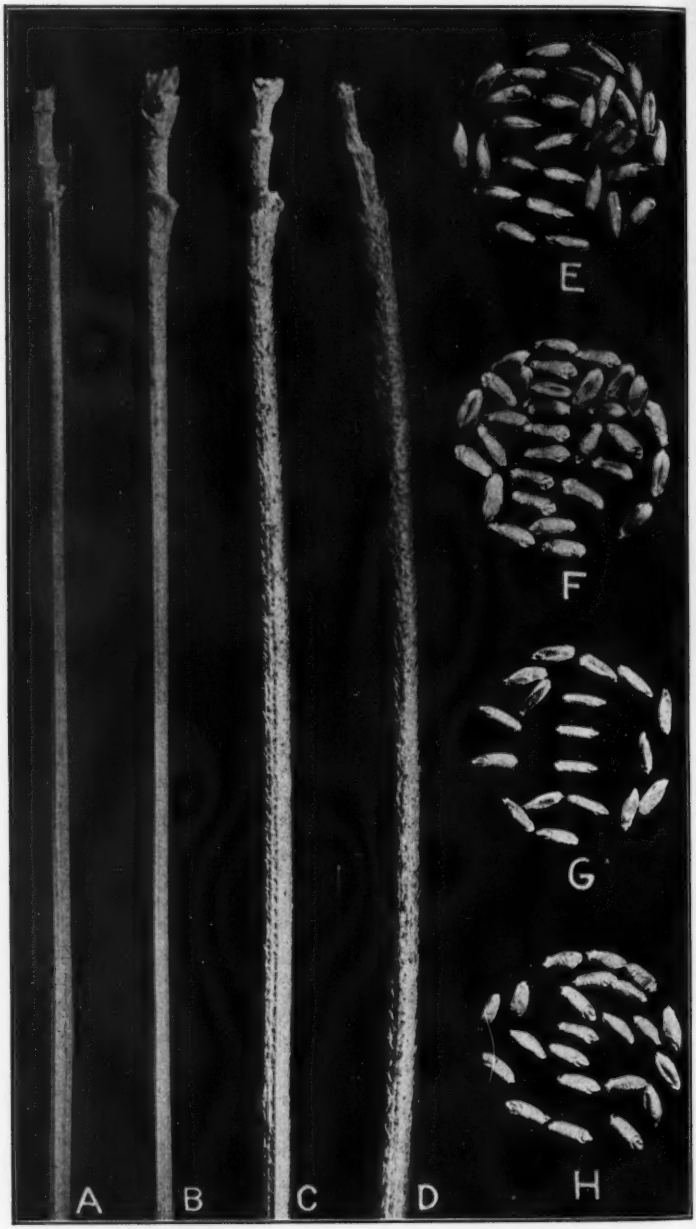

A
B
C
D
E
F
G
H

PLATE 2

Necks (upper portions of peduncles) of rye culms: A, glabrous; B, sparsely pubescent; C and D, densely pubescent. (×3) Kernels of rye, wheat, and of two different types of head in the progeny of selection K (see Pl. 5). E, Abruzzes rye; F, Fulcaster wheat; G, fusiform head type (3) in selection K; H, oblong head type (4) in selection K. (Natural size.)

PLATE 3

Heads of wheat and 3 wheat-like selections with hairy necks from the F_3 progeny of natural wheat-rye hybrids: 1, selection K; 2, selection H; 3, Fulcaster wheat; 4, selection C. (Natural size.)

A
B
C
D

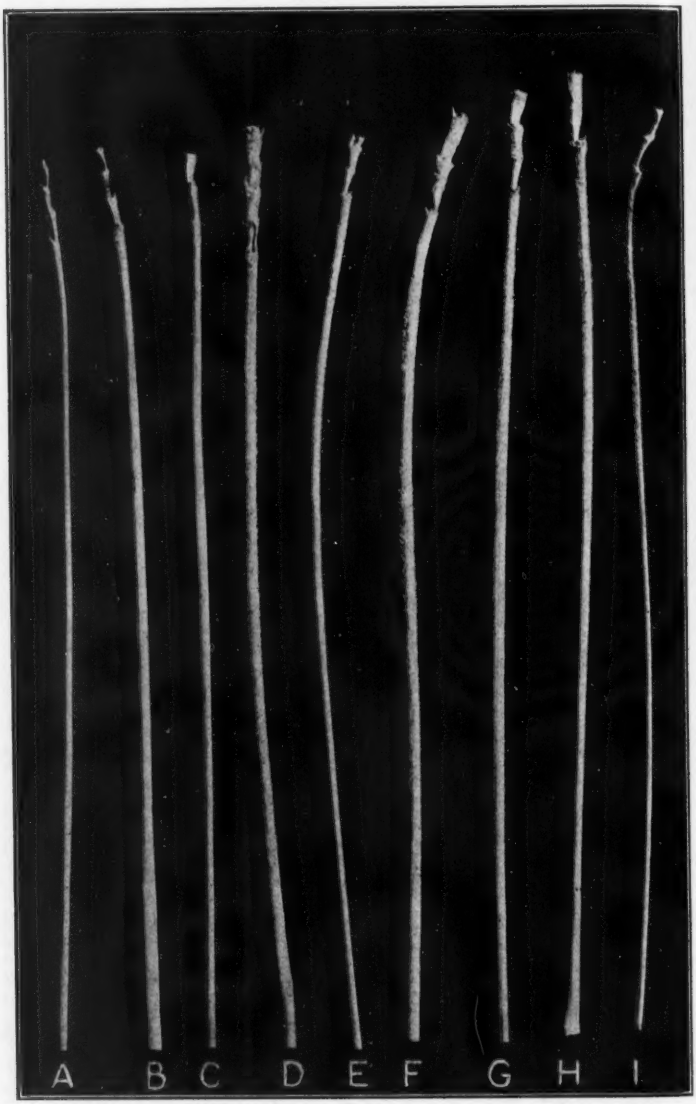

A
B
C
D
E
F
G
H
I

PLATE 4

Necks (upper portions of the peduncles) of the culms of wheat, rye, and selections from wheat-rye hybrids: 1, wheat; 2, selection K; 3, probable natural hybrid in progeny of selection 1; 6, selection D; 7, selection F; 8, selection C; 9, rye. (Natural size.)

PLATE 5

Head types present in the F_3 generation of wheat-rye hybrid plants with hairy necks: 1 and 2, probable natural hybrids found in progeny of selections I and K, respectively; 3 and 4, two types of heads in the progeny of selection K. Head No. 3 produced the rye-like kernels (G); and head No. 4 the wheat kernels (H) shown in Plate 2. (Natural size.)

A
B
C
D

ASCARIS SENSITIZATION [1]

B. H. RANSOM, *U. S. Bureau of Animal Industry;* W. T. HARRISON, *Hygienic Laboratory, U. S. Public Health Service;* and J. F. COUCH, *U. S. Bureau of Animal Industry*

It is a well established fact that *Ascaris lumbricoides*, the common intestinal roundworm of human beings and pigs, and other more or less closely related species parasitic in various animals, have toxic properties. This is clear not only from the nature of the symptoms in many cases of infestation which can be explained only on the basis of a toxic origin but also from the experience of many persons, mostly laboratory workers, who have had occasion to handle these parasites or who have in some way been brought into contact with them. Zoologists, for example, who have made extensive use of the horse Ascaris in cytological studies, have been frequent sufferers from its poisonous effects, Goldschmidt (1910) who was himself susceptible discovered by inquiry among zoologists of his acquaintance about 20 cases of similar susceptibility. (*2*).[2] Prior to Goldschmidt various writers, Bastian, Huber, Railliet, Linstow, and others, had reported personal experiences with the toxic effects of Ascaris, involving either the horse Ascaris or the human Ascaris. In fact susceptibility to Ascaris toxins is a very common condition among biologists who have been much exposed to contact with the parasite. Thus Ascaris sensitization is a sort of occupational disease. Its significance in relation to the toxic action of Ascaris in cases of infestation with the parasite remains to be determined.

The symptoms in persons who are sensitive to contact with Ascaris are of a kind that immediately suggest a similarity to the symptoms seen in cases of hay fever, asthma and other conditions grouped under the heading of foreign protein sensitization. Among the symptoms are irritation of the mucous membranes of the eyes, nose, and throat, lacrimation and edema of the eyes, facial edema, sneezing, coughing, swelling of the nasal mucosa, increased nasal and bronchial secretions, painful deglutition, urticaria, asthma, headache, fever, pruritus, tingling and burning sensations, swelling of the fingers, lassitude and weakness, sometimes amounting to prostration. In some cases the asthmatic attacks have persisted for weeks after exposure, but it is not certain in these cases that the possibility of subsequent exposures was entirely excluded. It has been commonly stated that the offending substance or substances are given off as emanations by the worm and thus are presumably volatile, but that they are actually volatile in the usual sense of the word has not been finally proved, although Weinberg and Julien (*3*) obtained a positive ophthalmic reaction in one out of 25 horses tested with the products of distillation of the body-cavity fluid of the horse ascarid, a result tending to support the prevalent belief in the presence of volatile substances in Ascaris that will produce symptoms in sensitive persons. Our own investigations thus far have failed to demonstrate a volatile substance that causes symptoms in human subjects sensitive to Ascaris, but we have not yet in our work taken up the question of those fractions of the worm that are insoluble in water.

[1] Received for publication May 12, 1924.

[2] Reference is made by number (italic) to "Literature cited," p. 582.

The horse Ascaris appears to be more powerful in its effects than the human Ascaris, judging from the fact that more severe symptoms have been commonly reported among persons sensitive to the former than among those sensitive to the latter, but this may be related to the fact that the horse Ascaris has been in general more intensively worked with by zoologists than the human Ascaris, so that exposure has commonly been greater in the case of the former. Individuals may be sensitive to both species, as in the case of Goldschmidt, or sensitive to one and not to the other, as in the case of Bastian, in whom the horse Ascaris would cause severe asthmatic attacks but upon whom the human Ascaris had no effect. Whether an individual may be sensitive to the human Ascaris and not to the horse Ascaris has not been clearly established although we have been informed that one zoologist who was susceptible to the effects of the human Ascaris, experienced no discomfort after this susceptibility had been noticed in handling a large number of living specimens of the horse Ascaris. As noted by both Bastian and Goldschmidt the severity of the symptoms if originally mild may increase with repeated exposures, and in some cases the symptoms have become so severe as to force susceptible individuals to abandon further scientific work involving the use of the offending parasites.

The investigations that we have recently undertaken are the outcome of the discovery by one of us of his susceptibility to the toxic action of *Ascaris lumbricoides* on contact with the worm, (4) a discovery made about five years ago. At that time following the appearance of eye symptoms (irritation, lacrimation, and swelling of the eyelids) a cutaneous scratch test was made with some of the body fluid of the parasite, the results of which were positive. Later on one occasion the same individual experienced a severe general reaction as the result of a drop of Ascaris fluid falling unnoticed on a very small but fresh abrasion of the skin. This attack was characterized not only by a local wheal with surrounding erythema, but by extensive lymphatic involvement, which was evidenced by red streaks extending up the arm to the shoulder, swelling of the face, urticarial wheals over the entire body, very rapid heart, discomfort in the throat, and a sensation of warmth and breathlessness. The more alarming symptoms disappeared within a couple of hours, but the face and eyelids were still swollen the following day and the arm in the region of the local reaction was still swollen and reddened. More or less discomfort and various unpleasant symptoms have since been experienced under circumstances that involved direct or indirect exposure to Ascaris, although care has been taken to minimize the chances of close contact with the parasite so far as is possible in a parasitological laboratory.

In our recent investigations, which are still unfinished and of which a complete report can not now be given, certain observations have been made which appear to be of sufficient interest to warrant the presentation of a brief progress report

About 20 persons, all white adults, have been tested for sensitiveness to *Ascaris lumbricoides* by means of a cutaneous scratch test. Among these there were 4 reactors, all of whom had been more or less exposed during periods of 5 to 25 years to contact with Ascaris. None of the reactors had a definite history of Ascaris infestation. Among the nonreactors were several who gave a history of having passed ascarids during childhood, and there were also several nonreactors who had at various times been considerably exposed to contact with Ascaris but had no recollection of unpleasant effects. Three of the reactors to the skin test had repeatedly experienced disagreeable symptoms on exposure to Ascaris; the fourth was previously unaware of his sensitiveness. In one of the cases the symptoms were asthmatic in type.

Different writers have reported that the characteristic odor of Ascaris may be detected on the breath of calves infested with *Ascaris vitulorum* and that the

meat of infested calves often smells so strongly of Ascaris as to render it unfit for food. In view of these reports the possibility naturally suggested itself that persons sensitive to Ascaris might also be similarly sensitive to pork from infested hogs if such meat contained toxic elements absorbed from the parasites infesting the animals. If it were found to be true that Ascaris-sensitive persons were sensitive to pork from infested hogs, the fact would be of great importance in its bearing on the question of pork sensitization, especially if at the same time such persons were insensitive to pork from noninfested hogs, inasmuch as a great deal of the pork that is eaten comes from Ascaris-infested hogs. Examination of about 2,500 hogs of various ages slaughtered in Chicago packing houses a few years ago showed an average of 41.1 per cent infested (*3*).

The following experiments were performed to determine whether pork from infested hogs contains a substance that will produce a skin reaction in persons sensitive to Ascaris. Aqueous, alcoholic, and ethereal extracts were made of pork from several infested hogs, and another similar series of extracts from a non-infested hog. Alcoholic and ethereal extracts were also made of fat from infested and noninfested hogs, respectively. None of these extracts gave a positive cutaneous scratch test in three Ascaris-sensitive individuals. Another experiment in which an aqueous extract of pork sausage was tested on six individuals, three of whom were sensitive to Ascaris, also gave negative results. The sausage used was a composite sample of sausage from several large meat-packing establishments. Any given lot of sausage manufactured in such establishments is likely to include trimmings from numerous hog carcasses, and in view of the frequent occurrence of Ascaris in swine the extract used in this experiment must have contained substances from the meat of a considerable number of infested hogs.

The results of these experiments, while not absolutely conclusive because of their negative character, indicate that the meat of Ascaris-infested hogs does not contain the substance that causes an urticarial skin reaction in Ascaris-sensitive individuals. In the light of this evidence there is no reason to suppose that Ascaris sensitization is involved in cases of pork sensitization.

Because of the writers' desire to learn something of the nature of the substance or substances in Ascaris that cause symptoms in susceptible persons, and, also, if possible, to isolate a substance that might appropriately be used to desensitize Ascaris-sensitive individuals, one of the writers undertook to separate various fractions of the constituent substances of the parasite. It was not considered advisable to attempt desensitization of human subjects with the crude substances of the worm inasmuch as certain pathological changes in the adrenals, thyroid, pituitary body, and pancreas of experimental animals have been recorded by other investigators following repeated injections of small quanties of the verminous material. Other investigators, also, notably Flury (1912), have reported the occurrence in Ascaris of a number of substances toxic to experimental animals. Flury (*1*) who has made the most extensive chemical and toxicological study of the parasite that has yet appeared has concluded that the toxic substances of Ascaris include aldehydes, free fatty acids, alcohols, esters, and two nitrogenous substances which are not protein in nature. He did not attempt to discover the substance which causes the urticarial skin reaction, and apparently was not familiar with this phenomenon. Shimamura and Fujii (1917) by fractionation of the watery extract from Ascaris separated a fraction which they termed "albumose-peptone" and to which they gave the name crude ascaron, applying the name ascaron to the inferred active principle, which they did not isolate (*5*). This ascaron they consider the active substance responsible for the toxicity of Ascaris, but their work throws little light upon the problem of the substance that causes the urticarial skin reaction.

Weinberg and Julien (*6*, *7*, *8*) have investigated the toxic action of the body-cavity fluid of the horse ascarid upon horses. The instillation of the fluid into the conjunctival sac was followed by a positive reaction in about two-thirds of 256 horses that were tested. This reaction which appears within a few minutes is characterized by edema of the eyelids, congestion of the conjunctiva, and lacrimation, occasionally accompanied by dyspnea, profuse sweating and diarrhea. Weinberg and Julien have concluded (*8*) that the toxicity of the fluid to sensitive horses is due to a number of active substances, inasmuch as they found the toxin to be thermostable, surviving exposure to a temperature of 120° C. for 20 minutes, filterable through a Chamberland filter, partially soluble in alcohol and in ether, and to contain volatile toxic constituents. The results that we have obtained in our investigations on human subjects with *Ascaris lumbricoides* are not entirely in accord with the conclusions expressed by Weinberg and Julien from the results of experiments with *Ascaris equorum* on horses. This discrepancy may be due not only to differences in the parasites and the experimental animals but also to the fact that Weinberg and Julien used body-cavity fluid of the worms in their tests while in our attempts to isolate an active substance we have been dealing with aqueous extracts of the worms and fractions of these extracts.

In our investigations swine ascarids (100 gm. in one lot and 1,774 gm. in another) after preliminary washing were ground up in a meat chopper, mixed with normal salt solution or 4 per cent ammonium sulphate solution and strained. The fluid portion was half saturated with ammonium sulphate and filtered. From the residue of this filtration a globulin fraction was obtained. The filtrate was precipitated by saturating with ammonium sulphate and after standing for 24 hours the albumens were collected by filtration. The filtrate was designated the protein-free filtrate for convenience although it still contained small amounts of proteins not precipitable by ammonium sulphate. It did not give the biuret reaction but gave a slight coagulum on heating. Three principal fractions were thus obtained from the original aqueous extract, a globulin fraction, an albumen fraction, and a so-called protein-free fraction. From the two latter various subsidiary fractions were prepared as shown in the list given below. Details of the chemical processes are reserved for a later report. The various fractions were tested on Ascaris-sensitive persons by means of the cutaneous reaction (scratch method). The results of these tests for each fraction are indicated by a plus or minus sign.

Fractions Nos. 1 to 4 were tested on three sensitive subjects, Nos. 5 to 9 on two, and Nos. 10 to 16 on one. In the test of fraction No. 8 the result was negative in the case of one subject and positive in the case of the other. The test was repeated in both cases with the same result, negative in one and positive in the other.

1. Globulin fraction	−
2. Albumen fraction	+
3. Protein-free filtrate (so-called)	+
4. Protein-free filtrate heated just to boiling	+
5. Protein-free filtrate separated into approximately equal parts by distillation. Distillate	−
6. Same. Residue	−
7. Protein-free filtrate after air had been drawn through it for 55 hours	+
8. Protein-free filtrate treated with potassium permanganate	− (+)
9. Precipitate from acidified protein-free filtrate by Mayer's solution	+
10. Filtrate from acidified protein-free filtrate after treatment with Lloyd's reagent	−
11. Material recovered in weak alkali from Lloyd's reagent after action on acidified protein-free filtrate	+
12. Filtrate from acidified albumen fraction after treatment with Lloyd's reagent	−
13. Material recovered in weak alkali from Lloyd's reagent after action on acidified albumen fraction	−
14. Filtrate from albumen fraction after treatment with 50 per cent alcohol	+
15. Filtrate from albumen fraction heated 17 minutes in boiling water	+
16. Albumen fraction digested 36 hours with pepsin	−

From the results of these experiments, assuming for convenience of discussion that only one substance is involved, it is evident that the substance in aqueous extracts of *Ascaris lumbricoides* that causes the skin reaction in Ascaris-sensitive individuals is absent from the globulin fraction, present in the albumen fraction and present in the so-called protein-free filtrate, after the removal of the globulin and albumen fractions by precipitation with ammonium sulphate. It is weakened by oxidation with potassium permanganate so that it no longer produces a skin reaction in all Ascaris-sensitive persons. It is not volatile at temperatures between 20° and 100° C. It is thermolabile and is destroyed by exposure to a temperature of about 100° C. for less than an hour but may survive exposure to a temperature as high as 100° C. acting for a period of about 15 minutes. It is destroyed in the albumen fraction by digestion with pepsin. It is soluble in 50 per cent alcohol. From the acidified protein-free filtrate it is wholly adsorbed by Lloyd's reagent and wholly precipitated by Mayer's solution. It is also wholly adsorbed from the acidified albumen fraction by Lloyd's reagent but has not been recovered from the latter by subsequent treatment with weak alkali, a procedure which releases it from Lloyd's reagent after adsorption from the so-called protein-free filtrate. The question whether it is of protein nature has not yet been answered by our investigations.

In conclusion reference may be made to experiments which one of us has made with the body-cavity fluid and aqueous extracts of *Ascaris lumbricoides*, and extracts of the dried and powdered worms on various animals, by means of injections and local applications to the skin and eye. So far as concerns the local reactions produced in experimental animals which appear to be in a measure comparable to the urticarial reaction in human beings it is of interest to note that individuals sensitive to *Ascaris lumbricoides* may be found among both hogs (Pl. 1) and sheep. Local reactions have not been observed in guinea pigs or rabbits, which are relatively resistant to the toxic effects of Ascaris substance when injected subcutaneously, intraperitoneally, or intravenously, whereas sheep and hogs, particularly the former, are highly susceptible to the toxic action of Ascaris substance when injected parenterally. Some dogs will react to dog ascarids brought in contact with the conjunctiva, and Weinberg and Julien (*6*, *7*, *8*) as already noted have shown that horses commonly give an ophthalmic reaction to the body-cavity fluid of the horse ascarid.

Although the substances in *Ascaris lumbricoides* which cause local reactions in hogs and sheep may prove to be different from the substance that causes the local skin reaction in sensitive human subjects the fact that reactions very similar to that occurring in the human subject can be produced in lower animals as conveniently available as hogs and sheep promises to be helpful in the investigation of the question of Ascaris sensitization and utilization of these experimental animals for studies on Ascaris sensitization may lead to results having an important bearing on the general problem of sensitization of human beings to foreign substances.

SUMMARY

(1) Human beings are commonly sensitive to a substance contained in aqueous extracts of the nematode, *Ascaris lumbricoides*, an intestinal parasite of man and the pig.

(2) Sensitive individuals exhibit a positive skin reaction when this substance is applied to a scratch on the skin, similar to that exhibited in cutaneous tests for so-called foreign-protein sensitization.

(3) This substance is apparently absent from the meat of Ascaris-infested hogs and there is no reason to suppose that it is involved in cases of sensitization to pork.

(4) Cutaneous tests on sensitive human subjects made with various fractions of an aqueous extract of Ascaris material separated by chemical and physical

means have shown that the substance in this extract that causes the skin reaction is absent from the globulin fraction.

(5) It is present in the albumen fraction, and is also present in the filtrate after precipitation of the albumen and globulin fractions by ammonium sulphate.

(6) It is weakened by oxidation with potassium permanganate.

(7) It is not volatile at temperatures between 20° and 100°C.

(8) It is thermolabile and is destroyed by exposure to a temperature of about 100°C. for less than an hour, but may survive exposure to a temperature as high as 100°C. acting for a period of about 15 minutes.

(9) It is destroyed in the albumen fraction by digestion with pepsin.

(10) It is soluble in 50 per cent alcohol.

(11) From the filtrate obtained after precipitation of the globulin and albumen fractions with ammonium sulphate it is wholly adsorbed in the presence of acid by Lloyd's reagent, and wholly precipitated by Mayer's solution.

(12) It is also wholly adsorbed from the acidified albumen fraction by Lloyd's reagent but has not been recovered from the latter by subsequent treatment with weak alkali, a procedure which releases it from Lloyd's reagent after adsorption from the aqueous extract from which the albumen and globulin fractions have been removed by precipitation with ammonium sulphate.

(13) The question whether the substance in *Ascaris lumbricoides* that causes the skin reaction in sensitive human subjects is of protein nature has not been answered by the investigations herein reported.

(14) A substance or substances that cause local reactions in some hogs and some sheep, comparable to the reactions observed in human subjects, are present in *Ascaris lumbricoides*. The ophthalmic reactions observed in these animals are altogether similar to those heretofore observed by Weinberg and Julien following the instillation of the body-cavity fluid of *Ascaris equorum* into the eyes of horses.

LITERATURE CITED

(1) Flury, F.
1912. zur chemie und toxikologie der ascariden. Arch. Exp. Path. u. Pharmakol. 67: 275–392.

(2) Goldschmidt, R.
1910. die askarisvergiftung. München. Med. Wchnschr. 57: 1991–1993.

(3) Ransom, B. H., and Foster, W. D.
1920. observations on the life history of ascaris lumbricoides. U. S. Dept. Agr. Bul. 817, 47 p., illus.

(4) ———
1922. observations on the toxic effects of ascaris fluids. Jour. Parasitol. 9: 42–43.

(5) Shimamura, T., and Fujii, H.
1917. ueber das askaron, einen toxischen bestandteil der helminthen besonders der askariden und seine biologische wirkung. (mitteilung I). Jour. Col. Agr. Imp. Univ. Tokyo 3: 189–258, illus.

(6) Weinberg, M., and Julien, A.
1911. substances toxiques de l'ascaris megalocephala. recherches expérimentales sur le cheval. Compt. Rend. Soc. Biol. [Paris] 70: 337–339.

(7) ——— ———
1911. exemple d'immunité acquise vis-à-vis d'une toxine vermineuse. Compt. Rend. Acad. Sci. [Paris] 152: 1030–1032.

(8) ——— ———
1913. recherches sur la toxine ascaridienne. Hyg. Viande et Lait 7: 225–244, illus.

PLATE 1

Ophthalmic reaction in a pig following the application of a few drops of Ascaris fluid to the conjunctiva. This animal also showed marked general symptoms in addition to the local eye reaction—vomiting, frothing at the mouth, frequent defecation, panting, and depression. The photograph was taken about 20 minutes after the application of the fluid.

PHYSALOSPORA MALORUM ON CURRANT[1]

By NEIL E. STEVENS

Pathologist, Fruit-Disease Investigations, Bureau of Plant Industry, United States Department of Agriculture

A fungus having pycnidia and spores similar to those of *Sphaeropsis malorum* Berk. was reported on dead canes of cultivated currant (*Ribes* sp.) more than twenty years ago by Grossenbacher and Duggar.[2] These writers, however, made no suggestions as to its life history or specific identity. From the information now available it is apparent that the perfect stage of this organism is a Physalospora, and that the fungus must be regarded as identical with *P. malorum* (Berk.) Shear (*P. cydoniae* as used by Hesler) the cause of black rot of the apple (*Pyrus malus*). This fact is of pathological interest, for obviously the problem of disease control is complicated when a fungus of economic importance on one host occurs on various unrelated hosts. Morphological and cultural comparisons have proven also that the difference in the size and appearance of the fruiting structures of the fungus on the two hosts is due to differences in the substratum. The taxanomic significance of this is apparent, for once it is proven, as in the present case, that a readily observable morphological character of a fungus is not constant but varies according to the nature of the host upon which it is found, such a character can no longer be accepted as a basis of generic or specific segregation.[3]

REVIEW OF EARLIER WORK

The suggestion that the apple blackrot fungus may occur on hosts other than those closely related to apple is of course not new. In 1899 Paddock[4] proved by inoculation that Sphaeropsis sp. from such unrelated hosts as *Diospyros virginiana* L., *Rhus typhina* L., *Celastrus scandens* L., and *Sambucus canadensis* L., will cause an apparently typical "blackrot" of apples. Paddock further maintained that the average size of the pycnospores varied in many cases according to the host on which they grew, yet the spores produced on apple fruits inoculated with cultures from these hosts were usually of the same size and character. Hesler[5] gives a long list of hosts, including Ribes, on which *Sphaeropsis malorum* has been collected and cites cross inoculation experiments with material from fourteen hosts.

As noted above, Grossenbacher and Duggar[6] report finding on currant canes a "Sphaeropsis-like fungus" which they refer to the genus Haplosporella in their early studies of currant cane blight.

Received for publication April 26, 1924.

[2] GROSSENBACHER, J. G., and DUGGAR, B. M. A CONTRIBUTION TO THE LIFE-HISTORY, PARASITISM, AND BIOLOGY OF BOTRYOSPHAERIA RIBIS. N. Y. State Agr. Exp. Sta. Tech. Bul. 18, p. 184. 1911.

[3] Many of the slides on which the morphological part of this paper is based were prepared by Rhoda Benham during the years 1919 and 1920, the rest by Ruth Colvin in 1923 and 1924. Much of the culture work was done by Marguerite Wilcox. The photomicrographs were made by Miss Colvin.

[4] PADDOCK, W. THE NEW YORK APPLE-TREE CANKER. N. Y. State Agr. Exp. Sta. Bul. 163, p. 194. 1899.

[5] HESLER, L. R. BLACKROT, LEAF SPOT, AND CANKER OF POMACEOUS FRUITS. N. Y. Cornell Agr. Exp. Sta. Bul. 379, p. 95-98. 1916.

[6] GROSSENBACHER, J. G., and DUGGAR, B. M. OP.CIT.

During the course of the present study of this disease which has extended over the last six years the writer has frequently found this Sphaeropsis on dead currant canes, often in close association with *B. ribis* or its form, *B. ribis chromogena*. It was not, however, until the summer of 1923 that mature perithecia of this fungus were found on currant and the identity of this fungus with *Physalospora malorum* established by morphological and culture studies.

ASSOCIATION OF THE PYCNIDIAL STAGE OF PHYSALOSPORA MALORUM WITH BOTRYOSPHAERIA RIBIS ON CURRANT CANES

Dead currant canes, whether killed by cane blight or not, frequently bear mature pycnidia of *Physalospora malorum*. Mature pycnidia of *P. malorum* often occur in close association with both *B. ribis* and *B. ribis chromogena*, the pycnidia of which as explained in an earlier paper [7] are of the Dothiorella type. On currant canes, moreover, the superficial appearance of the fruiting bodies of the fungi is somewhat similar. So closely, indeed, are the fungi associated and so similar their general appearance that during the early stages of our work pycnidia of the Sphaeropsis type frequently occurred in sections prepared for microscopic study of the currant cane blight fungus.

These considerations together with the fact that for several years only one type of ascospore was found on currant suggested the possibility that the "Sphaeropsis-like" pycnidia might be a stage in the life history of the Botryosphaeria or at least that the Sphaeropsis might be parasitic on currant. Inoculation experiments on currants with cultures made from single spores of the Sphaeropsis from currant and from other hosts gave, however, uniformly negative results. This was true even under conditions which gave striking infections when the true parasite, *Botryosphaeria ribis chromogena* was used. These results agree with those of Grossenbacher and Duggar.[8] Moreover, although several hundred single spore cultures of the fungi from currant have fruited, no connection could be established between the pycnidia of the Sphaeropsis type and either spore form of *B. ribis*. These facts made it appear highly improbable that the Sphaeropsis was a pycnidial form of the Botryosphaeria. Satisfactory proof of the distinctness of the two fungi was, however, not obtained until the summer of 1923 with the completion of the morphological and culture studies on Physalospora and Botryosphaeria on apple already reported [9] and the discovery of the ascogenous stage belonging to the Sphaeropsis on currant.

THE PERFECT STAGE OF THE SPHAEROPSIS ON CURRANT

Although the perfect stage of the cane blight fungus as well as the saprophyte *B. ribis*, has frequently been collected during the past six years and hundreds of single spore cultures have fruited, ascospores which produced pycnospores of the Sphaeropsis type in culture have been found only once on currant. This was at North Rochester, Mass., where, on June 27, 1923, a single currant cane covered with mature perithecia was found in a pile of old currant prunings. This material was at once recognized by Miss Wilcox, who was at that time carrying on culture studies of the currant cane blight fungus and related fungi at Woods Hole as different from *B. ribis* and very similar to *Physalospora malorum*. Thirty-seven

[7] SHEAR, C. L., STEVENS, N. E., and WILCOX, M. S. BOTRYOSPHAERIA AND PHYSALOSPORA ON CURRANT AND APPLE. Jour. Agr. Research 28: 589–598, illus. 1924.

[8] GROSSENBACHER, J. G., and DUGGAR, B. M. A CONTRIBUTION TO THE LIFE-HISTORY, PARASITISM, AND BIOLOGY OF BOTRYOSPHAERIA RIBIS. N. Y. State Agr. Exp. Sta. Tech. Bul. 18, p. 148. 1911.

[9] SHEAR, C. L., STEVENS, N. E., and WILCOX, M. S. BOTRYOSPHAERIA AND PHYSALOSPORA ON CURRANT AND APPLE. Jour. Agr. Research 28: 589–598, illus. 1924.

single ascospore culture from this material all produced pycnospores indistinguishable from those of *Sphaeropsis malorum*. Subsequent investigation showed the fungus to be apparently identical with *Physalospora malorum*. The specimen here discussed has been deposited in the pathological collections of the Bureau of Plant Industry, United States Department of Agriculture.

COMPARISON OF THE PHYSALOSPORA FROM CURRANT AND FROM APPLE

The first characteristic which suggested that the material in question was related to Physalospora rather than Botryosphaeria was that the ascospores germinated by the single long unbranched germ tube characteristic of Physalospora and never, in the writer's experience, found in *B. ribis*. In cultural characters this fungus also agreed with cultures of *P. malorum* from apple on all the media tested and was readily distinguishable from *B. ribis* on such media as beef agar, potato agar, and corn meal in flasks.

The comparative size and shape of the spores from the two hosts is shown in Table I. The ascospores measured were taken direct from the host. The pycnospores, on the other hand, were grown in pure culture from single ascospores, as this seems to be the only method of making sure of the actual connection of the two spore forms. The close similarity in size and shape of the spores from the two hosts is apparent This agreement is more marked in the case of the pycnospores, which were grown in pure culture under similar conditions, than in the case of the ascospores which were taken from the hosts. Whether this circumstance is significant and indicates either that the size of the ascospores is slightly modified by the host, as suggested by Paddock for the pycnospores, or that the form found on currant is actually slightly different from that on apple can not now be determined. This point could be settled only by the production of ascospores in quantity in pure cultures, or on the host under sterile conditions. Neither of these lines of attack is possible with our present knowledge.

TABLE I.—*Spores of Physalospora malorum*

ARRANGED BY CLASSES ACCORDING TO LENGTH

	Length (microns)																					
	Total	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
Ascospores on apple	223	1	---	1	---	---	5	8	4	11	10	16	11	31	20	28	24	30	11	6	3	2	---	1
Ascospores on currant	146	---	---	1	---	4	8	9	15	9	30	15	12	21	4	6	6	3	2	---	---	1	---	---
Pycnospores in culture from ascospores on apple	100	---	1	5	1	18	17	14	20	11	9	---	3	1	---	---	---	---	---	---	---	---	---	---
Pycnospores in culture from ascospores on currant	100	1	1	13	5	14	13	16	11	15	5	1	3	1	---	1	---	---	---	---	---	---	---	---

ARRANGED BY CLASSES ACCORDING TO WIDTH

	Width (microns)										
	Total	6	7	8	9	10	11	12	13	14	15
Ascospores on apple	223	------	25	31	46	68	26	23	3	1	------
Ascospores on currant	146	1	2	14	23	41	33	22	9	------	1
Pycnospores in culture from ascospores on apple	100	------	------	4	8	36	25	17	8	2	------
Pycnospores in culture from ascospores on currant	100	------	------	6	8	36	30	11	5	2	3

TABLE I.—*Spores of Physalospora malorum*—Continued

ARRANGED ACCORDING TO RATIO OF LENGTH TO WIDTH

	Ratio (microns)								
	Total	1.5	2	2.5	3	3.5	4	4.5	5
Ascospores on apple	223	-----	6	46	77	58	21	11	4
Ascospores on currant	146	1	27	59	40	13	5	1	-----
Pycnospores in culture from ascospores on apple	100	3	42	51	4	-----	-----	-----	-----
Pycnospores in culture from ascospores on currant	100	8	41	46	3	2	-----	-----	-----

RELATION OF SIZE AND STRUCTURE OF SPOROCARPS TO HOST

The only striking difference in the fungi on the two hosts is in the size and structure of the sporocarps and this the writer believes to be due to the effect of the substratum. Plate 1, A, shows in vertical section a typical mature perithecium of *Physalospora malorum* on apple, while Plate 1, B and C, show mature perithecia of *P. malorum* on currant. Usually on currant several perithecia are grouped together in a single sporocarp, as shown in Plate 1, B, and these are surrounded by and supported on a mass of stromatic tissue. Even when, as occasionally happens, a perithecium occurs singly on currant it will almost invariably be partly embedded in a mass of stromatic tissue.

A similar difference is found in the pycnidia. On apple the pycnidia usually occur singly and without any noticeable amount of stromatic tissue (Plate 1, D), whereas on currant the pycnidial cavities are usually grouped in large stromata (Plate 1, G, and Plate 2, A), or when they do occur singly are partly surrounded by stromatic tissue (Plate 1, F).

The presence or absence of a stroma has been considered of sufficient importance to separate species or even genera and families. That it can not be so considered in the case now under consideration is abundantly proven by the fact that the presence of a stroma in the fungus on currant is not a constant character. If a pure culture of the fungus from currant is permitted to fruit on a portion of sterile apple twig, the resulting pycnidia will mostly occur singly and will have little, if any, associated stromatic tissue. The same thing is true if such a culture fruits on any twig having a smooth bark with fine texture. Plate 1, E, for example, shows a typical pycnidium of the currant fungus grown in pure culture on a sterile portion of a black raspberry cane (Rubus). On the other hand, when the fungus from apple is grown on currant the condition is reversed, and the sporocarps are large and usually contain several spore cavities.

The experimental evidence that the size of the sporocarp is directly influenced by the host has reference at present merely to the pycnidial stage because we are not yet able to produce ascospores in quantity in culture. There is no reason to suppose, however, that the same relation would not hold for the perfect stage. In nature ascospores and pycnospores occur in similar sporocarps and on currant were found to occur in the same sporocarp.

Attention has already been called to the fact that the amount of stromatic tissue about pycnidia and perithecia of *Botryosphaeria ribis* is directly affected by the host upon which it grows.[10] In the case of this fungus as in Physalospora a larger ascocarp is formed when the fungus fruits on currant than when it fruits on twigs having a thinner and more closely knit bark. Whether this difference

[10] STEVENS, N. E., and JENKINS, A. E. OCCURRENCE OF THE CURRANT CANE BLIGHT FUNGUS ON OTHER HOSTS. Jour. Agr. Research 27: 837–844, illus. 1924.

is due to differences in the physical character of the bark or to the chemical composition of the food the twigs supply to the fungus must remain for further investigation.

That the change in amount of stromatic tissue and in arrangement of spore cavities is in fact due to the host is further indicated by the behavior of the closely related *Diplodia natalensis* Pole-Evans, which causes a serious rot of citrus fruits in Florida.

Plate 2, B, C, and D, show sections of mature pycnidia of *Diplodia natalensis* grown on sterile twigs of apple and currant from single spore cultures from grapefruit. It will be noted that on apple twigs the pycnidia are usually separate even when near together, whereas on currant twigs they tend to aggregate. The difference here must be due to the difference in substratum, since the cultures were all made at the same time by transfers from a single spore culture and during their growth on twigs were kept together in flasks on the same bench in a greenhouse for the same length of time. This would seem to indicate that in these fungi the size of the sporocarps and the amount of stromatic tissue associated with a single spore cavity is not a constant character.

SUMMARY

The perfect stage of the Sphaeropsis common on dead currant canes has been demonstrated to be a Physalospora, which is apparently identical with *P. malorum*, the fungus causing blackrot of apples. The chief difference in the appearance of the fungus on the two hosts is in the size of the sporocarps, which on currant are large and usually contain several spore cavities, while on apple the sporocarps are much smaller and usually contain only one spore cavity. This difference in the size of the sporocarp is apparently due to the difference in the substratum. Pycnidia produced on sterile apple twigs from pure cultures of the currant fungus are small, while those produced on sterile currant twigs from pure cultures of the apple fungus are large and usually contain several spore cavities. A similar difference in the size of the sporocarps is found when pycnidia of *Diplodia natalensis* are produced in pure cultures on sterile twigs of apple and currant.

PLATE 1

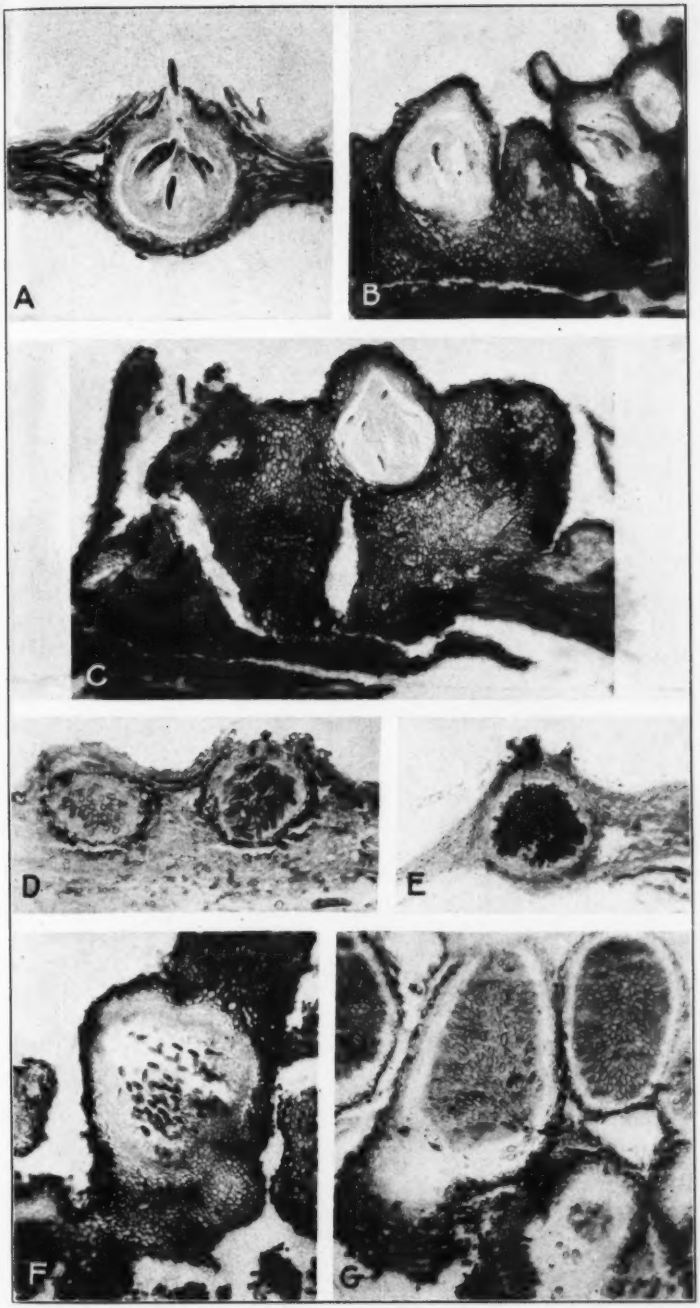
A.—Mature perithecium of *Physalospora malorum* on apple bark.

B, C.—Portions of sporocarps of *P. malorum* on currant bark; containing mature perithecia.

D.—Pycnidium of *P. malorum* on apple bark.

E.—Pycnidium of *P. malorum* on black raspberry from a pure culture from currant.

F, G.—Portions of sporocarps of *P. malorum* on currant containing mature pycnidia.


A
B
C
D
E
F
G

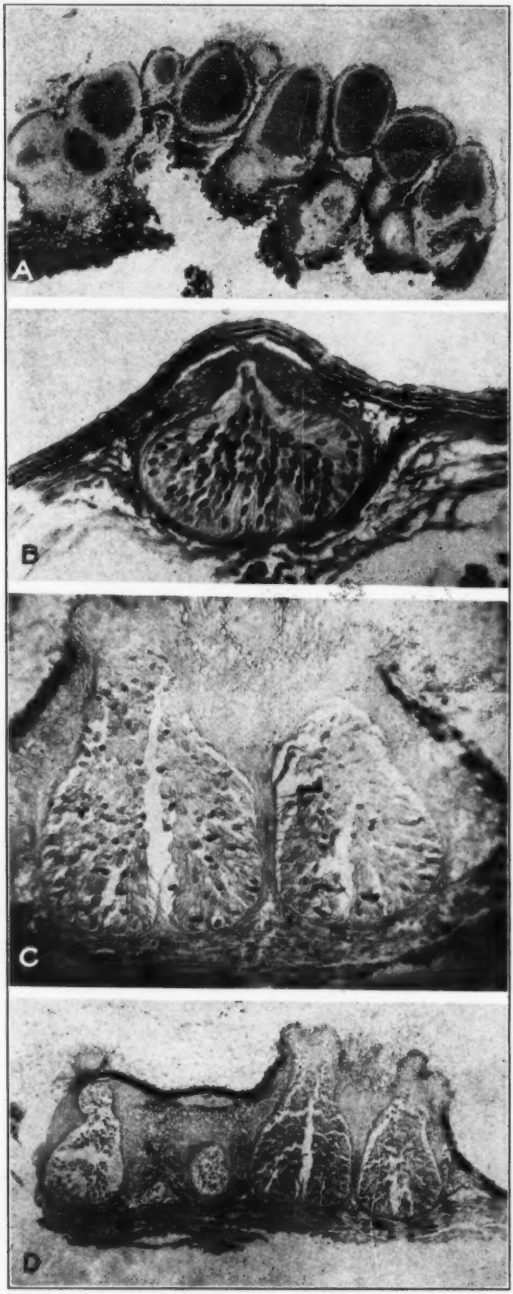

A
B
C
D

PLATE 2

A.—Sporocarp of *P. malorum* on currant. × 45.

B.—Sporocarp of *Diplodia natalensis* from grapefruit produced on apple twig in pure culture. × 90.

C.—Portion of a sporocarp of *D. natalensis* from grapefruit produced on currant twig in pure culture. × 90.

D.—Sporocarp of *D. natalensis* from grapefruit produced on currant twig in pure culture. × 45.

BOTRYOSPHAERIA AND PHYSALOSPORA ON CURRANT AND APPLE[1]

By C. L. Shear and Neil E. Stevens, *Pathologists*, and Marguerite S. Wilcox, *Laboratory Aid, Office of Fruit-Disease Investigations, Bureau of Plant Industry, United States Department of Agriculture*

The economic importance of black rot of apple (*Pyrus malus*) and cane blight of currant (*Ribes rubrum*) seems to make advisable a statement of our present knowledge regarding their causal organisms even though our investigation of this group of fungi has not reached a point which warrants a detailed account of the synonomy of these species or of their life histories, genetic relationships, and distribution on the 26 hosts from which closely related fungi have already been collected and critically studied. The information at present available indicates that the ascogenous stages of these fungi are so similar as to suggest their very close relationship, and that both forms of *Botryosphaeria ribis*[2] occur on apple, on which host at least one form seems to be parasitic and capable of producing a fruit rot under some circumstances.

REVIEW OF EARLIER WORK

The life history of the currant cane blight fungus was worked out by Grossenbacher and Duggar (*2*)[3] who also established its parasitism. To this fungus they gave the name *Botryosphaeria ribis*, distinguishing between the parasite and a purely saprophytic form which occurred commonly on currant, by the fact that the parasite developed a "purplish pink color" when grown on starch paste, while the saprophyte, which was morphologically identical with the parasite, developed no such color.

The literature on the black rot of apples and its causal organism is voluminous. Until 1913, however, it dealt almost exclusively with the pycnidial form of the fungus which was usually referred to as *Sphaeropsis malorum*. In December of that year, both Hesler (*4*) and Shear (*10, 14, p. 107*) published the results of pure culture studies which established the relationship of *Sphaeropsis malorum* to its ascogenous stage. This perfect stage Hesler referred tentatively to *Physalospora cydoniae* Arnaud, while Shear repeated his suggestion made three years earlier (*9*) in describing the life history of what is apparently the same species on grape, that it was *Melanops quercuum* (Schw.) Rehm forma *vitis* Sacc. or a variety of this species.

Three years later Shear and Beckwith (*11*) announced that pycnospores of the *Sphaeropsis malorum* type had been produced in pure culture from single ascospores from a variety of hosts including apple, and that pycnospores of the type of the currant cane blight fungus, which they refer to as a Dothiorella, have

[1] Received for publication Feb. 6, 1924.

[2] The name Botryosphaeria is used here for species congeneric with *Botryosphaeria ribis*, Grossenbacher and Duggar, as represented by specimens in Fungi Columbiani No. 3409 labeled *B. ribis achromogena*, Grossenbacher and Duggar. As thus defined the genus comprises part of the species included by Cesati and de Notaris (*1*) in their original description of this genus. This name is adopted instead of Melanops because of general usage. Fungi Columbiani No. 3408 represents *B. ribis chromogena* of this paper.

[3] Reference is made by number (italic) to "Literature cited," p. 598.

also been produced from single ascospores from apple, and other hosts. No suggestions are made as to the taxonomic status of these fungi, other than that they are closely related and that some of them have previously been referred to as forms of *Melanops quercuum*.

In 1919 Putterill (*6*) described a canker of apple trees in South Africa which was caused by a fungus closely resembling *Botryosphaeria ribis*. This fungus even possessed the chromogenesis (*6*, p. 264) described by Grossenbacher and Duggar for the currant parasite. The characters used by Putterill to distinguish his fungus, for which he proposes the name *Botryosphaeria mali*, are a difference in the width of the asci and in the size of the stromata. Recently Stevens and Jenkins (*15*) have demonstrated that the currant cane blight fungus occurs on horsechestnut, and certain varieties of rose, and that it is parasitic on the rose, causing cankers on the stem and sometimes killing whole canes.

LIFE HISTORY STUDIES

Culture Methods.—During the last two years the writers have had under observation over three thousand cultures of fungi belonging to this group, more than ninety per cent of which have fruited. In view of the difficulty experienced by some investigators in obtaining pycnospores of these fungi in pure cultures, a brief statement of the methods used may be of interest. It is certainly true that these fungi fruit but rarely in the arid and often superheated environment furnished by many laboratories. If, however, the cultures are kept in an ordinary greenhouse the temperature of which varies through a range from 50° to 70° F. or more, they will fruit abundantly on many agar media, on sterile twigs, and on cornmeal in flasks. Summer temperatures in the vicinity of Washington, D. C., are apparently too high to permit these fungi to fruit readily but good results have been obtained during the summer in unheated wooden buildings at Wareham and Woods Hole, Mass.

Our cultures were first transferred to the greenhouse in an endeavor to secure more variable temperatures, as a result of the observations of Stevens (*12*), recently emphasized by Harvey (*3*), that the temperature of bark out of doors often fluctuates with great rapidity. There is as yet, however, no certainty that the abundant fruiting is due to temperature range or fluctuation alone. It may as well be due to the greater humidity, or to a combination of temperature and humidity, or to some as yet unrecognized and incidental factor. It is sufficient for the purpose of the present investigation to be able to secure abundant pycnospore production with reasonable certainty in pure cultures made either from mycelial transfers or from ascospores or pycnospores.

Results.—In the course of this culture work the writers have verified the life histories recorded above many times. Within the last two years alone pycnidia of the Dothiorella type have matured in pure culture from one hundred eighty single ascospores from currant and from seventy-seven single ascospores from apple. Mature pycnidia of the Sphaeropsis type have been produced in pure culture from ninety-five single ascospores from apple.

The name Dothiorella is here used to include such pycnidial forms as are congeneric with the macro-pycnidial stage of *Botryosphaeria ribis* and apparently also *Dothiorella gregaria* Sacc. Typical material is found in specimens 3407, 3408, and 3409, Fungi Columbiani. This is not *Dothiorella ribis* (Fuck.) Sacc. Number 3407 labeled "Macrophoma type" is a mere form of Dothiorella growing on young shoots and producing smaller and simpler pycnidia. The name Macrophoma has been most frequently applied to immature forms of Diplodia and Sphaeropsis, in which the spores are colorless. The name Sphaeropsis is here used to include pycnidial forms congeneric with the form usually called *S. malorum* on apple and whose ascogenous stage is Physalospora.

RELATIONSHIP OF THE FUNGI HAVING PYCNIDIA OF THE DOTHIORELLA TYPE

The collection and growth in pure culture of so large a quantity of material for a study of the life histories of the fungi, and especially the fact that a very large proportion of the cultures fruited in less than two months from the time they were made, has enabled us to make a more careful comparison of the fungi concerned than has hitherto been possible. Although the study has included material from a variety of hosts and from widely separated localities it is proposed to discuss here only the forms which are known to occur on apple and those described by Grossenbacher and Duggar on currant.

The writers' studies of the currant cane blight fungus and its saprophytic form have extended over a period of six years and have included the more important currant-producing regions of the United States. In general, the careful work of Grossenbacher and Duggar has been fully confirmed. The parasitism of the form which is characterized by the production of a bright pink color on starchy media has been confirmed by inoculation experiments in Virginia, both outdoors and in the greenhouse, as well as in Connecticut and Massachusetts. Inoculations with the non-chromogenic form have given uniformly negative results.

Continued collecting has, as might be expected, resulted in finding that the ascospores vary through a somewhat wider range than that given by Grossenbacher and Duggar, the great majority of the spores, however, fall within the limits set by them namely, ascospores, 16–23 μ by 5–7 μ, and pycnospores 16–31 by 4.5–8 μ. Moreover, it is apparent that the size of the stromata varies very widely and is dependent to some extent on the thickness of the bark within which

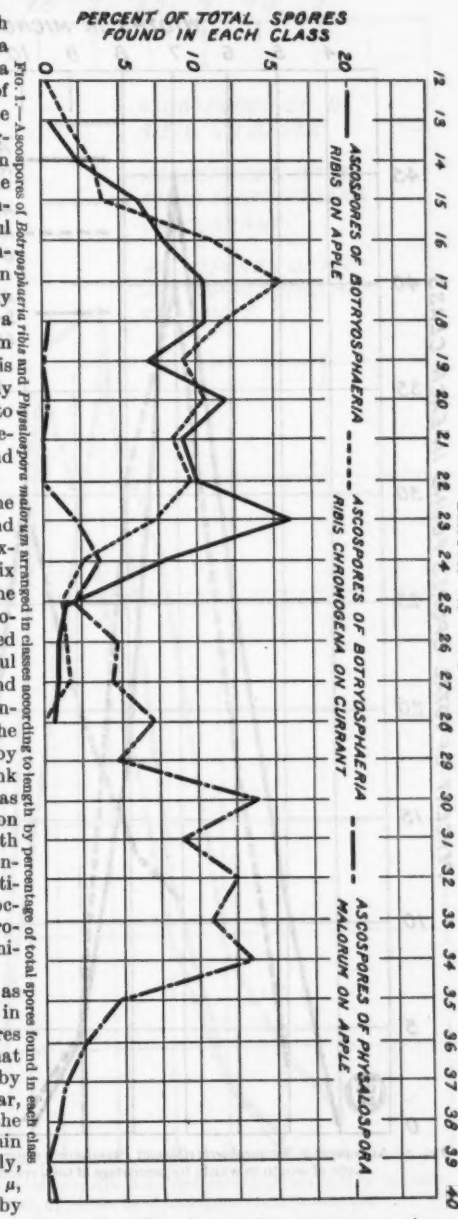

PERCENT OF TOTAL SPORES FOUND IN EACH CLASS
0
5
10
15
20
LENGTH IN MICRONS
12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40
ASCOSPORES OF BOTRYOSPHAERIA RIBIS ON APPLE
ASCOSPORES OF BOTRYOSPHAERIA RIBIS CHROMOGENA ON CURRANT
ASCOSPORES OF PHYSALOSPORA MALORUM ON APPLE

FIG. 1.—Ascospores of *Botryosphaeria ribis* and *Physalospora malorum* arranged in classes according to length by percentage of total spores found in each class

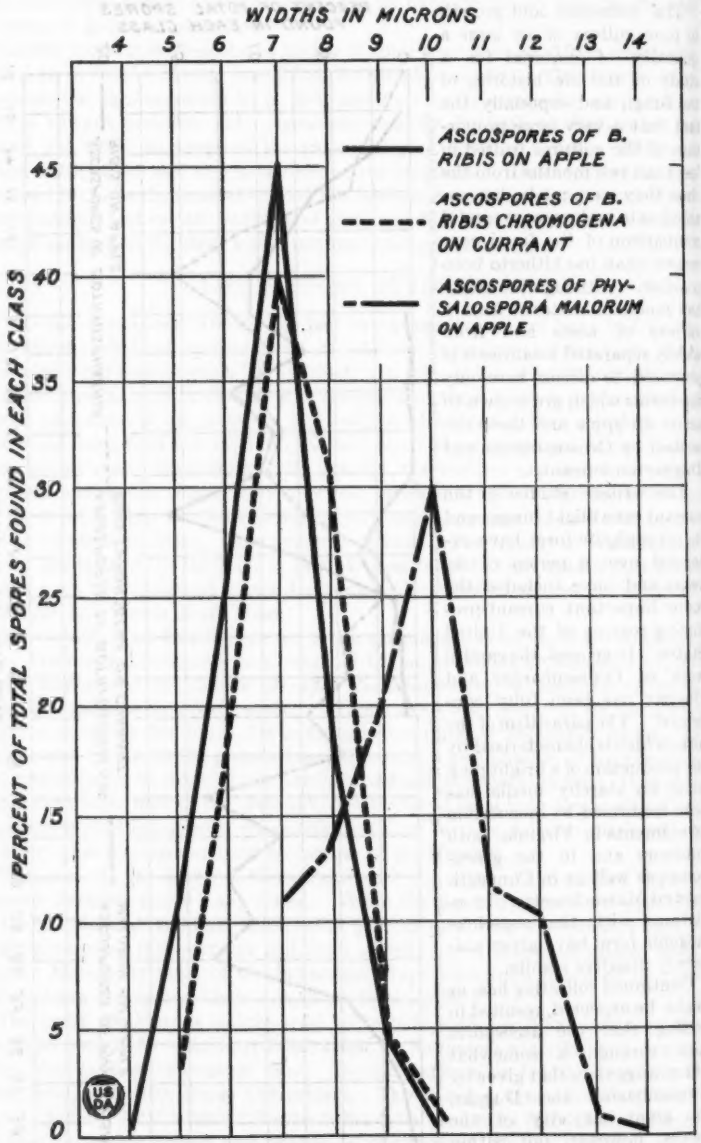

WIDTHS IN MICRONS
4 5 6 7 8 9 10 11 12 13 14
PERCENT OF TOTAL SPORES FOUND IN EACH CLASS
0 5 10 15 20 25 30 35 40 45
ASCOSPORES OF B. RIBIS ON APPLE
ASCOSPORES OF B. RIBIS CHROMOGENA ON CURRANT
ASCOSPORES OF PHYSALOSPORA MALORUM ON APPLE
U.S. D.A.

FIG. 2.—Ascospores of *Botryosphaeria ribis* and *Physalospora malorum* arranged in classes according to ratio of length to width, by percentage of total spores found in each class

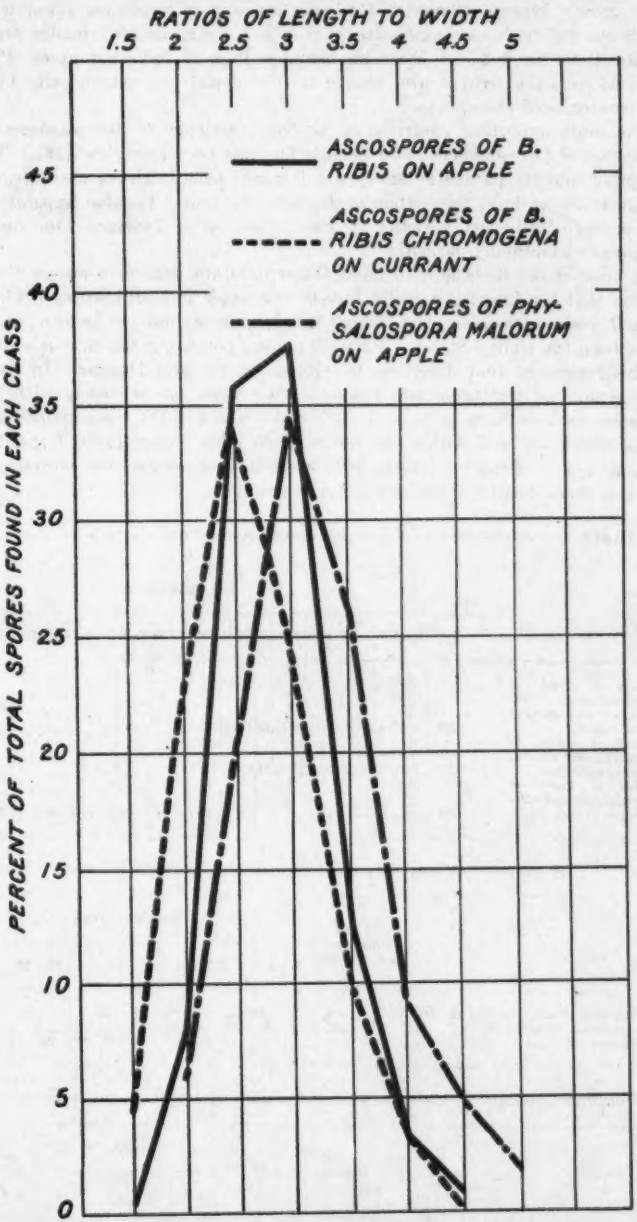


FIG. 3.—Ascospores of *Botryosphaeria ribis* and *Physalospora malorum* arranged in classes according to width, by percentage of total spores found in each class

they grow Mature stromata of *B. ribis chromogena* have been observed on a single currant bush varying in size from 1 × 2–3 mm. on the smaller branches (Plate 1, *C*) to 3–3.5 × 5–8 mm. near the base of the older canes (Plate 1, *D*). As yet, the writers are unable to distinguish morphologically between the two forms of this species.

The most important contribution to our knowledge of *Botryosphaeria ribis* since that of Grossenbacher and Duggar has just been published (*13*). This is the proof that the parasitic variety which causes cane blight of currants, occurs in nature on at least two other hosts, horsechestnut (*Aesculus hypocastanum*) and several cultivated varieties of rose (*Rosa* sp.). Moreover, on rose this fungus is undoubtedly parasitic.

In view of the facts now in hand the writers are unable to escape the conclusion that the fungus found by Putterill on apple in South Africa is identical with *B. ribis chromogena* even though this fungus has not yet been reported on apple from the United States. Putterill himself points out the close resemblance of this fungus to that described by Grossenbacher and Duggar. In size and arrangement of perithecia and pycnidia his fungus agrees closely with theirs; the ascospores he finds to be 19.2–19.5 × 6.5–8 μ, and the pycnospores 32.4 × 4.8 μ, which are well within the limits of *B. ribis*. (See Table I and figures 1, 2, and 3.) Moreover, (*6*, p. 264) his fungus possessed the chromogenesis which is characteristic of the parasite on currant.

TABLE I.—*Ascospores arranged by classes according to length in microns*

	Total number of spores	Class (in microns)																										
		13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
Number of spores of *Botryosphaeria ribis chromogena* on currant in each class	226	5	7	9	25	35	27	21	24	19	22	16	6	3	3	4	---	---	---	---	---	---	---	---	---	---	---	---
Number of spores of *Botryosphaeria ribis* on apple in each class	207	1	5	13	8	22	22	14	25	19	21	33	16	3	2	2	1	---	---	---	---	---	---	---	---	---	---	---
Number of spores of *Physalospora malorum* on apple in each class	223	---	---	---	---	---	1	---	1	---	---	5	8	4	11	10	16	11	31	20	28	24	30	11	6	3	2	---	1

Ascospores arranged by class according to width in microns

	Total number of spores	Class (in microns)											
		4	5	6	7	8	9	10	11	12	13	14	15
Botryosphaeria ribis chromogena on currant	226	---	8	44	91	70	11	2	---	---	---	---	---
Botryosphaeria ribis on apple	207	1	11	54	94	37	10	---	---	---	---	---	---
Physalospora malorum on apple	223	---	---	---	25	31	46	68	26	23	3	1	---

Ascospores arranged by class according to ratios of length to width in microns

	Total number of spores	Class							
		1.5	2	2.5	3	3.5	4	4.5	5
Botryosphaeria ribis chromogena on currant	226	10	50	78	56	22	8	---	2
Botryosphaeria ribis on apple	207	1	17	74	78	26	7	2	2
Physalospora malorum on apple	223	---	6	46	77	58	21	11	4

The chief difference in the fungi, according to Putterill, lies in the fact that the asci of his form from apple are narrower than those measured by Grossenbacher and Duggar and the stromata of the apple fungus are usually about 0.5 mm. wide as contrasted with 2 mm. as the most common size on apple. Neither of these characters appears to the present writers to have significance. The asci are variable in width and are frequently found in material from currant no wider than those noted by Putterill. The stromata of this fungus, as already pointed out, vary in size with the thickness of the bark in which they grow. Mature perithecial stromata of *B. ribis* on rose are often 0.3 mm. or less in diameter, yet this fungus has been proven by inoculation experiments to be the same as that producing much larger stromata on currant.

That the character of the bark in which they are produced directly influences the size of the stromata in this species seems to be fully proven by their artificial culture on sterile twigs from different species of woody plants. Plate 1, *A* and *B*, show mature pycnidial stromata which developed on dormant apple and currant twigs from subcultures of *B. ribis* from currant. These cultures were started at the same time and kept near together in special culture flasks on a bench in a greenhouse from November 19, 1921, to February 22, 1922. A glance at the figures will show the relative size of the stromata on the two hosts. To be sure these are pycnidial stromata as the writers are not yet able to produce mature perithecia in quantity in pure culture. Under natural conditions, however, perithecia are often produced in the same stromata with pycnidia and on any given host the stromata bearing the two kinds of spores are of the same size.

Unfortunately the writers have not been able to secure cultures of the fungus from Africa for inoculation on currant, although Putterill has courteously tried to secure more living material for this work. Under date of January 25, 1921, he writes as follows:

> "I have just returned from a visit to the infected trees at Vereeninging. Since my first visit there the trees have been more carefully treated, the diseased areas having been cut out and applications of coal tar made at regular intervals; I was unable to obtain fresh material for you."

In spite of the fact that inoculations can not be made, there seems to be no ground for considering the African fungus different from that which produces the cane blight of currants. Mere distance between the localities can not be considered a reason for considering the fungi different, especially when they occur on cultivated plants which are known to be shipped and carry their parasites long distances.

As to the possibility of this fungus being parasitic on apple in this country there is no evidence at present. Putterill's letter would suggest that it is sometimes not very virulent in South Africa since he was unable to find it in the orchard in which he had previously found it. That it can be made to grow on apple tissue is evident from the result of the inoculations made by Putterill on sound apples. Some years ago while checking up the results of shipping experiments on citrus for Rogers and Earle (*8*) the writers inoculated sound grapefruit with the currant cane blight fungus and produced a rot somewhat resembling the Diplodia stem end rot.

Since *B. ribis chromogena* is an active parasite on currant, Stevens and Jenkins were able to prove the identity of the material which they collected from horse-chestnuts and rose with the cane blight fungus by actual inoculation experiments. This type of proof is not possible in the case of the saprophytic or nearly saprophytic form. There is, however, no reason to consider the nonchromogenic Botryosphaeria which occurs on apple in this country as specifically distinct from the non-chromogenic saprophytic Botryosphaeria on currant, since no morphological differences have been found. (See Table I, text figures 1, 2, and 3, and Plate 2, A, B, C, and K-N).

The practice of describing as "new species" saprophytes or omnivorous parasites having practically identical morphological characters simply because they happen to be found on different hosts or in a distant locality, is to be deplored as it cumbers mycological literature with a mass of doubtful names and synonyms and greatly impedes the progress of mycological taxonomy. Real differences in essential morphological characters must be the foundation for specific segregation. What "real" and essential differences are can only be determined by careful and thorough study of a considerable amount of good material of the organisms in hand. Moreover when it has been shown by cross inoculations or studies of the fungi in pure culture that the characters upon which species have been segregated are such as result from host or cultural conditions and vary with a change of these conditions, they must be considered specifically identical.

COMPARISON OF BOTRYOSPHAERIA RIBIS AND PHYSALOSPORA MALORUM

Physalospora malorum is here used for the fungus previously referred to as *Melanops quercuum* forma *vitis* by Shear (*9*) and *Physalospora cydoniae* Arnaud by Hesler (*4*), the pycnidial form of which is *Sphaeropsis malorum*. As there appear to be no good specimens of this species in published American exsiccati the writers will distribute what they regard as typical material of both stages to the principal large herbaria in the near future. The name *Physalospora malorum* is chosen as a combination of the best known names of the two stages of the fungus.

Both perithecia and pycnidia of *Botryosphaeria ribis* usually occur in a stroma, though single perithecia are often found and the size of the stromata varies, as has been pointed out above, with the host, and thickness of the bark. Hesler, in describing *Physalospora cydoniae*, the perfect stage of *Sphaeropsis malorum* (*5*), states that "the perithecia are usually scattered, standing separate from one another. Sometimes, however, from two to four fruit bodies are joined together, but no stroma has ever been observed." The writers have not yet made a sufficiently extended study and comparison of these characters to form an opinion as to their constancy and taxonomic value.

The paraphyses in both these species are very characteristic and have never, so far as we know, been correctly described or illustrated. They appear to consist of a tangled or anastomosing mass of septate, filamentous hyphae. In young perithecia the central portion is filled with a mass of pseudo-parenchyma. As the asci develops they push up into this mass, which becomes more or less filamentous at maturity, but separate paraphyses have never been observed.

The most striking difference in the perfect stages of the two organisms is the size of their ascospores. See text figures 1, 2, and 3, Table I, and Plate 2. As is evident from the Table and figures the longer spores of *B. ribis* equal in length the shorter ones of *Physalospora malorum* so that from a single spore it is not always possible to distinguish the two fungi. The great majority of ascospores of *P. malorum*, however, are longer than the largest yet measured from *B. ribis*. and in good preparations of mature ascospores the two species are easily distinguished on this basis. The same relation holds with regard to the width of the ascospores of the two fungi. The wider ascospores of *B. ribis* equal the narrower ascospores of *P. malorum* but as a whole the ascospores of *P. malorum* averaged about 3 μ wider. In shape as expressed by the ratio of length to width the ascospores of the two fungi are very similar indeed. See figure 3 and Table I.

The mode of liberation and ejection of ascospores has been observed in *Physalospora malorum* in fresh mature specimens mounted in water. The wall of the ascus ruptures transversely and the ascospores are ejected while still imbedded in a somewhat gelatinous matrix, having the same outline as the ascus and extending to the base where it appears to be attached, as illustrated in Plate 2, *F*. and *G*.

Micropycnospores are found in culture and nature associated with the macropycnospores of both the Sphaeropsis and Dothiorella types. They may occur in separate locules or be intermingled with the macropycnospores. They are minute in size, about 2–3 μ long, oblong, and have never been observed to germinate. See Plate 2, figure N.

An interesting and apparently constant difference between the ascospores of the two fungi is found in their method of germination. Ascospores of *Botryosphaeria ribis*, whether from currant or from apple, characteristically develop two germ tubes which branch before they have reached more than 8 or 10 times (Pl. 2 *O.* and *P.*) the length of the spore. Even where only one germ tube is produced it usually branches while rather short. Ascospores of *Physalospora malorum*, on the other hand, usually develop only one germ tube which rarely if ever branches before it reaches a length equal to 50 or 60 times that of the spore itself. (Pl. 2, *Q.* and *R.*) So constant under the conditions of our work was this apparently trivial character that in the case of two hundred eighty-six ascospores of these two fungi in which the type of germination has been observed and the development of the pycnospores in pure culture subsequently obtained, those ascospores which germinated by means of a single, long, unbranched germ tube have always produced pycnospores of the Sphaeropsis type and those which germinated by means of the shorter branched germ tubes, usually two to a spore, have always produced pycnospores of the Dothiorella type.

On many culture media early stages of the development of the two fungi appear much alike, especially when grown in dry air at temperatures above 22° C. They may be readily distinguished, however, on beef agar made according to the following formula:

Add 3 gm. of beef extract, 10 gm. peptone, and 5 gm. of sodium chlorid to one liter of distilled water. Steam one hour; titrate with hydrochloric acid and make up to plus 10, Fuller's scale. Add enough water to make one liter, and 1½ per cent shredded agar. Steam one hour. After cooling to 60° C. add the whites of two eggs well beaten—steam another hour, filter through cotton, tube and autoclave 20 minutes at 15 pounds pressure.

Cultures made on agar slants of this medium from mycelial transfers show after ten days a loose felt of short, rather fluffy mycelium on the surface of the medium. *Botryosphaeria ribis*, however, leaves the color of the medium unchanged (Baryta yellow) (7) whereas *Physalospora malorum* changes the medium to a dark color, between mummy brown and black.

Cultures on corn meal in 100 cc. Erlenmeyer flasks which have reached the stage of producing mature pycnospores are readily distinguished by the surface character of the fungus growth. Fruiting or nearly mature cultures of *Botryosphaeria ribis* show numerous raised knob-like stromatic bodies usually 2 to 3 mm. wide and 3 to 4 mm. high in which the pycnidia are contained. (Pl. 1, *E.*)

Fruiting cultures of *Physalospora malorum* on corn meal in flasks on the other hand have a much more uniform surface without prominent regular elevations of any kind, the pycnidia being almost completely buried in the mycelial growth. (Pl. 1, *F.*)

If the various differences in stromata, size of ascospore, method of germination, cultural characters, and different pycnidial stages mentioned above are constant, they furnish a good basis for generic segregation. The two generic names which have commonly been applied to the two species on these hosts are here used for convenience and clearness.

SUMMARY

The life histories of *Botryosphaeria ribis*, causing cane blight of currant, and *Physalospora malorum*, causing black rot of apple, have been verified repeatedly by the development of the pycnospores from single ascospores in pure culture.

The methods by which pycnospores of these fungi were readily produced in pure culture are briefly described.

The differences between *Botryosphaeria* and *Physalospora*, as the names are used here, are the apparent difference in life histories, the first having Dothiorella as its pycnidial stage and the second Sphaeropsis as its pycnidial stage and the difference in ascospore sizes.

The ascogenous stages of *Botryosphaeria ribis* and *Physalospora malorum* may be distinguished by size of ascospore, method of germination of ascospores, and by certain cultural characters.

The fungus on apple in Africa described as *Botryosphaeria mali* by Putterill is apparently identical with the physiological variety of *Botryosphaeria ribis* G. and D. which the writers call *chromogena*.

The fungus commonly found on apple in this country which has Dothiorella as its pycnidial stage and closely resembles that from Africa in morphology, but is nonchromogenic, is apparently identical with *Botryosphaeria ribis* G. and D.

LITERATURE CITED

(1) Cesati, V., and Notaris, G. de
1863. schema di classificazione degli sferiacei italici aschigeri più o meno appartenenti al genere sphaeria nell' antico significato attribuitogli da Persoon. Comm. Soc. Crittog. Ital., 1: 177–287 b.

(2) Grossenbacher, J. G., and Duggar, B. M.
1911. a contribution to the life history, parasitism and biology of botryosphaeria ribis. N. Y. State Agr. Exp. Sta. Tech. Bul. 18, p. 115–190, illus.

(3) Harvey, R. B.
1923. cambial temperature of trees in winter and their relation to sun scald. Ecology 4: 261–265.

(4) Hesler, L. R.
1913. physalospora cydoniae. Phytopathology 3: 290–295, illus.

(5) ———
1916. black rot, leaf spot, and canker of pomaceous fruits N. Y. Cornell Agr. Exp. Sta. Bul. 379, p. 49–148, illus.

(6) Putterill, V. A.
1919. a new apple tree canker. So. African Jour. Sci. 16: 258–272, illus.

(7) Ridgway, R.
1912. color standards and color nomenclature. 43 p., illus. Washington, D. C.

(8) Rogers, J. M. and Earle, F. S.
1917. a simple and effective method of protecting citrus fruits against stem-end rot. Phytopathology 7: 361–367.

(9) Shear, C. L.
1910. life history of melanops quercuum (schw.) rehm forma vitis sacc. (Abstract) Science 31: 748.

(10) ———
1914. life history of sphaeropsis malorum berk. (Abstract) Phytopathology 4: 48–49.

(11) ——— and Beckwith, A. M.
1916. life histories of melanops. (Abstract) Phytopathology 6: 109.

(12) Stevens, N. E.
1922. environmental temperatures of fungi in nature. Amer. Jour. Bot. 9: 385–390.

(13) ——— and Jenkins, A. F.
1924. occurrence of the currant cane blight fungus on other hosts. Jour. Agr. Research 27: 837-844, illus. 1924.

(14) Taylor, W. A.
1913. report of the chief of the bureau of plant industry. U. S. Dept. Agr. Ann. Rpt. 1913: 105–133.

PLATE 1

A.—Mature pycnidial stromata of *Botryosphaeria ribis* grown in a flask on portion of sterile apple twig in greenhouse from November 19, 1921, to February 22, 1922. ×2.

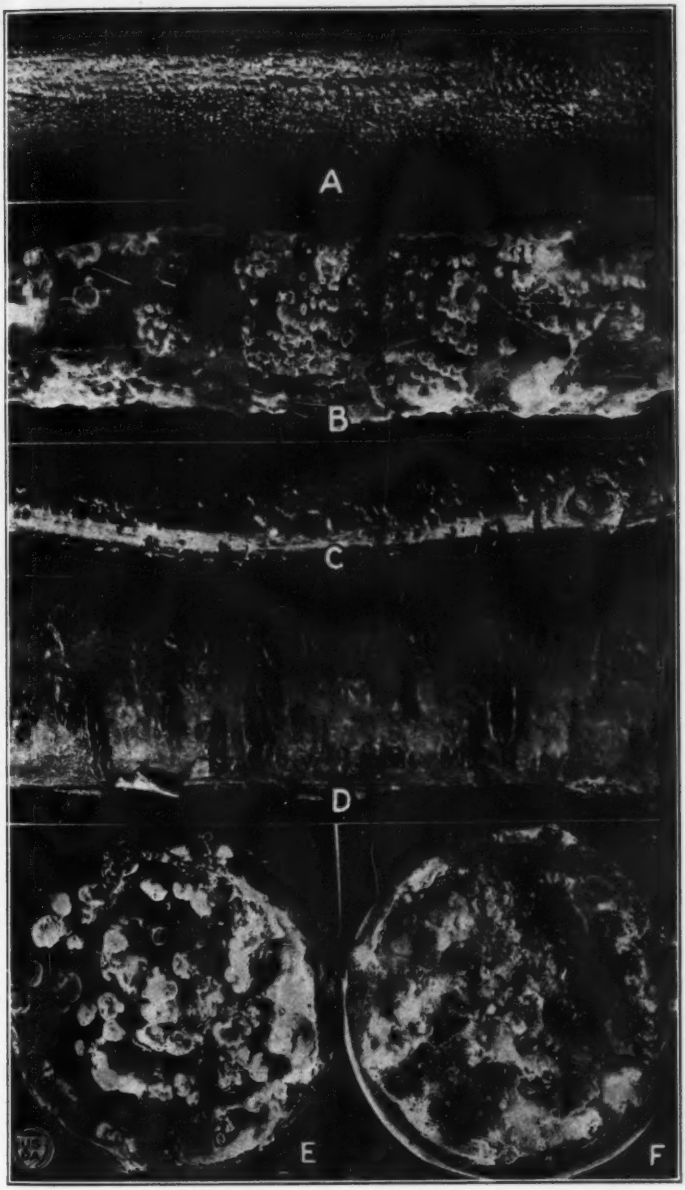
B.—Mature pycnidial stromata of *Botryosphaeria ribis* grown in a flask on portion of sterile currant twig in greenhouse from November 19, 1921, to February 22, 1922. ×2.

C.—Mature pycnidial and perithecial stromata of *Botryosphaeria ribis chromogena* on small branch of dead currant bush. North Rochester, Mass., June, 1921. ×2.

D.—Mature pycnidial and perithecial stromata of *Botryosphaeria ribis chromogena* on trunk of same bush as that shown in C. ×2.

E.—Fruiting culture of *Botryosphaeria ribis* on corn meal in flask.

F.—Fruiting culture of *Physalospora malorum* on corn meal in flask.


A
B
C
D
E
F

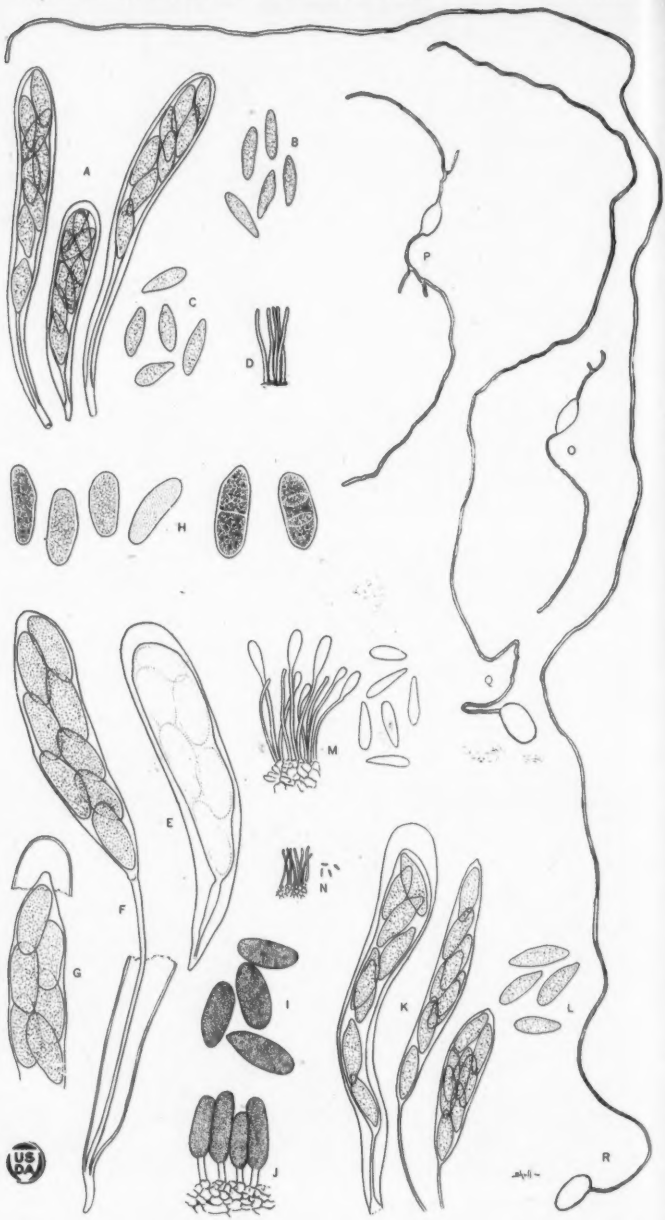

A
B
C
D
E
F
G
H
I
J
K
L
M
N
O
P
Q
R
USDA

PLATE 2

A.—Three asci of *Botryosphaeria ribis* on gooseberry, from host.

B.—Ascospores of *B. ribis* on gooseberry, from host.

C.—Macropycnospores of *B. ribis*, grown in pure culture from single ascospores from gooseberry.

D.—Sporophores of macropycnospores of *B. ribis* in culture.

E.—Ascus of *Physalospora malorum*, on apple, from host.

F.—Ascus of *P. malorum* showing mode of rupture and ejection of ascospores, from host.

G.—Upper portion of a ruptured ascus of *P. malorum* with part of the ascospores, from host.

H.—Ascospores of *P. malorum*, from host.

I.—Macropycnospores of *P. malorum* from single ascospores from apple, cultures 2581 and 2660 from Vienna, Va., and Ambler, Pa.

J.—Macropycnospores with sporophores from culture.

K.—Three asci of *Botryosphaeria ribis* on apple, from specimen 2805 collected at Vienna, Va.

L.—Ascospores of *B. ribis* on apple, from specimen 2805.

M.—Macropycnospores of *B. ribis* on apple, some attached to sporophores, from culture 3106 A. from a single ascospore.

N.—Micropycnospores and sporophores of *B. ribis* on apple, from culture 3106 A. Figures A to N. ×420.

O.—Germinating ascospore of *B. ribis* after 18 hours at a temperature of about 12° C.

P.—Same spore held four hours after the stage shown in O—at a temperature of 22° C.

Q.—Germinating ascospore of *Physalospora malorum* after 18 hours at a temperature of about 12° C.

R.—Same spore held four hours after the stage shown in figure 1, at a temperature of 22° C. Figures O to P. ×200.

ISOLATION OF AN INHIBITORY SUBSTANCE FROM PLANTS[1]

W. L. MALLMANN and CARL HEMSTREET, *Department of Bacteriology, Michigan Agricultural College*

INTRODUCTION

The isolation of lytic and inhibitory substances from animal sources by various investigators led the writers to a study of diseased plants in order to determine whether or not such substances were found in association with plant pathogens.

It seems needless to review the literature on bacteriophage since so many recent articles have included extended reviews. A number of investigators have found bacteriophage present in the intestines of man and animals in association with disease organisms. It is generally believed that bacteriophage develops under a diseased condition, adapts itself to the causative organism, and where a recovery results, it develops a high lytic power, causing a lysis of the causative organism.

A survey of the available literature failed to reveal any work of this nature on plant diseases; hence the present work.[2]

Soft rot of cabbage was selected for study, as it was easily obtained. A cabbage showing soft rot was placed in a large culture dish and allowed to decompose until a considerable quantity of liquid material had collected. At this point, loop dilution plates were made in order to isolate the rot-producing organisms. As the plates showed practically a pure culture, several colonies were fished and planted on agar slants. One of these cultures was used for all the work carried

TABLE I.—*The effect of the cabbage-rot filtrate on different organisms* [a]

Culture	Control	Amount of filtrate added		
		1 drop	2 drops	2 cc
B. coli	−	−	−	−
Do	−	−	−	−
B. carotovorus	−	−	−	−
Do	−	−	−	−
B. melonis	−	−	−	−
Do	−	−	−	−
Cabbage-rot organism	−	±	±	±
Do	−	±	±	±

[a] The following symbols are used in the tables: −, very strong turbidity; ±, slightly less turbidity; +, cloudy; ++, less cloudy; +++, nearly clear; ++++, clear.

out. The collected liquid was filtered through a filter paper, previously impregnated with diatomaceous earth. The clear filtrate was passed through a Berkefeld filter to remove all the bacteria present. The technique used was similar to that recommended by d'Hérelle.[3] The organism was isolated and in addition stock strains of *Bacillus carotovorus*, *B. melonis*, and *B. coli* were now inoculated into tubes of plain broth and incubated for one hour. Varying amounts of the

[1] Received for publication Mar. 10, 1924.

[2] This work was done in the spring of 1922. Since writing this paper, the following article has appeared: GERRETSEN, F. C., AND OTHERS. DAS VORKOMMEN EINES BAKTERIOPHAGEN IN DEN WURZELKNÖLLCHEN DER LEGUMINOSEN. Centbl. Bakt., (II) 60: 311–316, illus. 1923.

[3] D'HÉRELLE, F., THE BACTERIOPHAGE, ITS RÔLE IN IMMUNITY. . . . Tr. by G. H. Smith. 237 p., illus. Baltimore. 1922.

sterile filtrate were then added to these tubes, as shown in Table I. The results were obtained in 48 hours. The tubes of the cabbage-rot organisms were now filtered as before and the filtrate again added to fresh broth cultures, with the results shown in Table II.

As Tables I, II, and III indicate, the second transfer shows a marked improvement in the inhibition of the organism and the third transfer shows an even more decided improvement over the second. In other words, a marked development of the inhibitory substance resulted from invigoration by transplanting.

TABLE II.—*The effect of the cabbage-rot organism after one invigoration*

Culture	Control	Amount of filtrate added		
		1 drop	2 drops	2 cc.
B. carotovorus	−	−	−	−
Do	−	−	−	−
Cabbage-rot organism	−	+	+	+
Do	−	+	+	+

[a] The following symbols are used in the tables: −, very strong turbidity; ±, slightly less turbidity; +, cloudy; ++, less cloudy; +++, nearly clear; ++++, clear.

In the same manner a third set was prepared with the following results (Table III):

TABLE III.—*The effect of the cabbage-rot filtrate after two invigorations* [a]

Culture	Control	Amount of filtrate added		
		1 drop	2 drops	2 cc.
Cabbage-rot organism	−	+	++	++++
Do	−	++++	++	++++

[a] The following symbols are used in the tables: −, very strong turbidity; ±, slightly less turbidity; +, cloudy; ++, less cloudy; +++, nearly clear; ++++, clear.

This substance is called an inhibitory substance because the tubes containing the filtrate remained clear for 48 hours and then slowly showed growth. The control tubes, on the other hand, were turbid in 24 hours. In no case was any evidence of lysis apparent, all the tubes showing only a retardation of growth for at least 48 hours.

The type of growth appearing in the tubes containing the filtrate was very interesting. The tubes after 48 hours would show a thin pellicle on the surface with a clear liquid beneath and no sediment. Upon agitation, this pellicle would break into granules, which would sink to the bottom of the tube. Upon shaking the tubes, the granules would break up and a persistent turbidity would result. The control tubes, on the other hand, would show a persistent turbidity in 24 hours without pellicle or sediment. The filtrate apparently induced this change of growth to take place.

Unpublished work by one of the authors (Mallmann) showed exactly the same occurrence in broth with *Bacillus coli* where an inhibitory substance of like nature was added. Plates from tubes of cabbage-rot organism showed only the typical normal colonies. These differ from the plates of *B. coli* where two types were found, a normal colony and a so-called "rough" colony, as shown by Bergstrand.[4]

After repeated transfers, the inhibitory substance adapted itself to some extent to other related organisms. It inhibited *Bacillus spieckermani* and *B. carotovorus*

[4] Bergstrand, H. ON THE VARIATIONS OF BACTERIUM COLI. *Jour. Bact. 8: 173-192, illus. 1923.*

TABLE IV.—*The effect of the cabbage-rot filtrate after repeated invigorations*[a]

Culture	Control	Amount of filtrate added		
		1 drop	2 drops	2 cc.
Cabbage-rot organism	−	++++	++++	++++
Do.	−	++++	++++	++++
Bacillus spieckermani	−	−	−	++++
Do.	−	−	−	++++
Bacillus carotovorus	−	−	−	++++
Do.	−	−	−	++++
Potato-rot bacillus	−	−	−	−
Do.	−	−	−	−

[a] The following symbols are used in the tables: −, very strong turbidity; ±, slightly less turbidity; +, cloudy; ++, less cloudy; +++, nearly clear; ++++, clear.

TABLE V.—*The effect of heating the filtrate at 56° C. for 30 minutes*[a]

Culture	Control	Amount of filtrate added		
		1 drop	2 drops	2 cc.
Cabbage-rot organism	−	+	++	++++
Do.	−	±	±	±

[a] The following symbols are used in the tables: −, very strong turbidity; ±, slightly less turbidity; +, cloudy; ++, less cloudy; +++, nearly clear; ++++, clear.

TABLE VI.—*The effect of heating the filtrate at 63° C. for 30 minutes*[a]

Culture	Control	Amount of filtrate added		
		1 drop	2 drops	2 cc.
Cabbage-rot organism, unheated filtrate	−	++++	++++	++++
Do.	−	++++	++++	++++
Cabbage-rot, heated filtrate	−	−	−	−
Do.	−	−	−	−

[a] The following symbols are used in the tables: −, very strong turbidity; ±, slightly less turbidity; +, cloudy; ++, less cloudy; +++, nearly clear; ++++, clear.

where a large amount of filtrate was added, but refused to inhibit the potato-rot bacillus. A filtrate made two weeks later from these tubes failed to inhibit *B. spieckermani* and *B. carotovorus* but was still very active against the cabbage-rot bacillus.

RESISTANCE OF THE INHIBITORY SUBSTANCE TO HEAT

An active filtrate was heated to 56° C. for 20 minutes and then added as usual to the cabbage-rot bacillus.

The heating at 56° C. for 20 minutes decreases the activity of the inhibitory substance but it was still active enough to show partial inhibition in one case.

A second active filtrate was now heated to 63° C. for 30 minutes and added as usual to young organisms in broth.

This temperature caused a complete destruction of the inhibitory substance as shown in the above table. This checks very favorably with previous work by others working on similar substances isolated from animal sources.

CONCENTRATION OF THE INHIBITORY SUBSTANCE

An active filtrate was added to broth cultures of young cabbage-rot bacillus in dilutions starting with 1 to 10 and progressing by dilutions of 10 up through 12 dilutions, or to a final dilution of 1 to 1,000,000,000,000. The tubes of broth contained 9 cc. of broth. To the first tube was added 1 cc. of filtrate making a 1-to-10 dilution. Using the same pipette, 1 cc. was taken from this tube to the second, and so on through the set. All of these tubes showed inhibition through a dilution of 1 to 100,000,000,000.

After a period of several months, the above experiment was repeated, using higher dilutions. The dilutions this time were run up by dilutions of 10 to a final dilution of 1 to 1,000,000,000,000,000,000. The filtrate still showed inhibition at a dilution of 1 to 100,000,000,000,000,000. The last dilution failed to show any inhibition.

The dilution in which the inhibitory substance is active is extremely high, far higher than could be induced through any toxic material produced by the organism. There is but little doubt that the material isolated is comparable to inhibitory substances obtained from animal sources.

The organism upon which the inhibitory substance was active proved upon identification to belong to the fluorescent group rather than *Bacillus carotovorus*.[5] However, this organism does decompose cabbage. Experiments to determine its decomposing power showed a slow rotting, several weeks being required for complete liquefaction of the cabbage.

Filtrates made from the pure culture never showed the inhibitory substance nor did the culture of *Bacillus carotovorus*. This proves that the inhibitory substance must have come from the cabbage. Filtrates from normal cabbage failed to show the inhibitory substance, which indicates an association of the inhibiting substance with the disease-producing organism. This is similar to conditions in the intestinal tract of animals as demonstrated by d'Hérelle.[6]

CONCLUSIONS

An inhibitory substance was isolated from a rotten cabbage which was active on an organism obtained from the same cabbage.

The inhibitory substance became active against other soft rot-producing organisms but this activity was lost by further transplanting.

The inhibitory substance was not destroyed at 56° C. for 20 minutes, but was destroyed at 63° for 30 minutes, showing a sensitiveness to heat comparable to microorganisms and lytic substances isolated from animal sources.

The inhibitory substance was present in extremely large amounts, as indicated by its activity in high dilution. It was therefore probably not a toxic product of the organism.

Lytic and inhibitory substances are probably found in plants as in animals. It is hoped that this brief preliminary report will open up this field and show the extent of lytic and inhibitory substances in the plant world.

---

[5] The organism is approximately the same size and shape as *Bacillus carotovorus*. It occurs singly and is sluggishly motile; Gram-negative, no gas produced in dextrose, lactose, or saccharose broth. Plain broth becomes decidedly turbid in 24 hours with a slight pellicle appearing in six to seven days, at which time a yellowish green coloration appears near the surface. The agar slant shows an abundant white opaque growth with no discoloration of the medium. Gelatin is liquefied.

[6] D'HÉRELLE, F., THE BACTERIOPHAGE, ITS RÔLE IN IMMUNITY. . . . tr. by G. H. Smith. 287 p., illus. Baltimore. 1922.

TWO HITHERTO UNREPORTED DISEASES OF STONE FRUITS[1]

By C. C. LINDEGREN, *Field Assistant*, and DEAN H. ROSE, *Pathologist, Fruit Disease Investigations, Bureau of Plant Industry, United States Department of Agriculture*

BOTRYTIS ROT OF PEACHES

In August, 1923, during the inspection of peach shipments from California, on the Chicago market, numerous fruits were found which showed a rot that was apparently due to Botrytis. On nearly all of these fruits there was a scanty growth of white to gray mycelium, and on a few of them the characteristic spore clumps of Botrytis could be seen. The varieties found attacked were Elberta and Lovell.

Cultures made under aseptic conditions from the advancing edge of the rotten spots yielded a Botrytis which was apparently very similar to *B. cinerea*. Positive evidence of the pathogenicity of the organism isolated was obtained in an experiment carried out as follows: Ripe healthy peaches were immersed for two minutes in 50 per cent alcohol, then rinsed thoroughly with sterilized distilled water; four of the peaches were inoculated by wounding with a flamed needle and forcing mycelium into the wound, four others were inoculated by merely laying a fragment of mycelium on the uninjured peach surface. For controls, four peaches were used which had received no treatment other than sterilization, and four others which after sterilization had been merely wounded with a flamed needle. All four of these lots were placed in moist chambers and held at room temperature. Within three days all of the peaches in the two inoculated lots showed light brown rotten spots about 2 cm. in diameter; by the end of eight days the spots had reached a diameter of 10 cm. Cultures from these spots yielded a Botrytis which was apparently identical with the one used for inoculations.

Similar results from inoculations were obtained in several other experiments conducted in the same way. The controls in all of the experiments failed to develop rot of any sort.

The noteworthy fact which these experiments establish is that Botrytis is able to penetrate the uninjured skin of the peach and that when it does so it can produce rot as rapidly as if the fruit had been wounded.

In lesions caused by Botrytis, peach tissue, both skin and flesh, is firmer in texture and lighter brown in color than in lesions caused by either Sclerotinia or Rhizopus. The skin around the margin of the lesion slips easily under pressure from the finger somewhat as in Rhizopus rot, but the decayed tissue does not have the marked sour odor characteristic of Rhizopus rot. As a matter of fact it has no definite odor of any sort. So far as noted there is no tendency to wrinkle and mummify.

ALTERNARIA ROT OF CHERRIES

During the summer and fall of 1923 the most conspicuous, although probably not the most destructive, disease of cherries on the Chicago market was a brown decay with abundant olive green, sporulating, aerial mycelium. Bing sweet cherries from Yakima and Wenatchee, Washington, and Lambert sweet cherries from Emmett, Idaho, showed a large amount of this rot. It was also found on

[1] Received for publication Apr. 1, 1924.

Journal of Agricultural Research,
Washington, D. C.

Vol. XXVIII, No. 6
May 10, 1924
Key No. G-405

96036—24†——10

Windsor sweet cherries from northern Michigan and on Montmorency (sour) and Early Richmond (sour) cherries from southern Michigan. The symptoms on sour and sweet cherries are decidedly dissimilar.

On the sweet cherry the rot is characterized by a firm, brown, cone-shaped mass extending in toward the pit. This firm, brown mass is made up of dead host cells interpenetrated and apparently held together by the fungous mycelium. It is firmer than the surrounding tissue, and if the skin that covers it be carefully broken the affected tissue beneath can often be lifted out intact. The skin which overlies the affected area is covered with an olive-green growth of sporulating mycelium; sometimes, however, this sporulating layer is hidden under a mass of white, fluffy mycelium.

The symptoms on sour cherry are quite different. The only similarity, in fact is the olive-green layer of sporulating mycelium growing on the epidermis, and even this soon becomes soaked with juice and looks black and matted. In the early stages of the disease there is apparently so little penetration of the mycelium below the epidermis that the whole surface of a cherry may be covered with the olive-green growth before the flesh is decayed near the pit. When the tissue decay does take place, it is light brown and not nearly so firm as that in the sweet cherry. No fluffy growth of mycelium has been observed on sour cherries by the writers. In general, the disease on sour cherries is characterized by a decayed epidermis covered with a black, water-soaked mat of sporulating mycelium. The stem of the decayed cherry is almost invariably dead and often contains fungi apparently saprophytic, notably Cladosporium sp. and Botrytis sp.

The olive-green decay is caused by Alternaria sp., which apparently enters through wounds in the cherry. These wounds during 1923 consisted mainly of cracks, the cause of which is entirely problematical. It is possible they were rain cracks, though certain circumstances suggest that they may arise in transit. The question is one which needs further investigation. Numerous isolations were made from collected specimens and several different organisms were obtained, namely, unidentified species of Rhizopus, Penicillium, Botrytis, Mucor, Aspergillus, and Cladosporium. Most of these organisms proved pathogenic, but lesions like those described above, particularly those described for sweet cherries, were produced only by inoculations with the Alternaria obtained from diseased cherries. There is no definite proof that the species of Alternaria isolated from the two kinds of cherries are identical. Cross-inoculations have shown, however, that the Alternaria from sweet cherries is pathogenic to sour cherries, and vice versa.

Inoculation experiments were set up as follows: The stems, which were usually dead and contained various species of fungi, were clipped and the cherries were sterilized in a 1 to 1,000 solution of mercury bichlorid, or in 50 per cent alcohol, for 5 to 30 minutes. They were then rinsed in sterilized tap water and placed in large sterile Petri dishes. Inoculations were made either (1) by forcing mycelium from an agar culture of Alternaria into the flesh of the cherries with a sterile needle, or (2) by merely laying fragments of mycelium on the surface of uninjured cherries. Infection was obtained in each of several hundred inoculations made by the first method and always resulted in lesions like those described above; from these lesions, on both sweet and sour cherries, an Alternaria was reisolated which was apparently identical with the one used for inoculation. No infection resulted from inoculations made by the second method. Failure to obtain it is taken to mean that the fungus is unable to penetrate the uninjured skin of the fruit. No infection developed on the controls, which consisted of wounded and unwounded cherries previously sterilized in the manner already described. All experiments were conducted at room temperature.

SUMMARY

There has been found, on peaches shipped from California, a rot from which a fungus similar to *Botrytis cinerea* has been isolated. It is proved, by inoculation and reisolation, that this fungus is the cause of the disease. The inoculation experiments indicate that the fungus is able to penetrate the uninjured skin of the fruit.

There has been found on sweet cherries shipped from Michigan a rot from which a species of Alternaria has been isolated. There is no proof that the Alternaria from sour cherries is identical with that from sweet cherries, but cross-inoculation and reisolation have shown that both strains are pathogenic to both kinds of cherries. The inoculation experiments indicate that the fungus is unable to penetrate the uninjured skin of the fruit.

ADDITIONAL COPIES

OF THIS PUBLICATION MAY BE PROCURED FROM
THE SUPERINTENDENT OF DOCUMENTS
GOVERNMENT PRINTING OFFICE
WASHINGTON, D. C.

AT

10 CENTS PER COPY
SUBSCRIPTION PRICE, $4.00 PER YEAR (DOMESTIC)
$5.25 PER YEAR (FOREIGN)

▽